ON THE TRYPANOSOMES OF BIRDS.*†

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INTRODUCTION.

THE problem of the cultivation of the malarial parasites, obviously one of great importance, is as yet untouched. The appearance of Schaudinn's remarkable paper[85]‡ in the early part of 1904 seemed to assure its definite solution. For, if the conclusions arrived at by this eminent investigator were found to be correct, all that would be necessary would be to cultivate the trypanosome stages by means of the method which has proved so successful with *Tr. Lewisi*, *Tr. Brucei*, and *Tr. Evansi*, and the problem would be solved.

It is but fair to say that we entered upon this work with the fullest confidence in the correctness of the conclusions reached by

* Received for publication January 8, 1905.

† This paper will be followed, in the next number of this *Journal*, by a second in which the other hematozoa of birds will be considered. A summary of the main results arrived at was presented before the International Congress of Arts and Science at St. Louis, September 23, and before the Society of Experimental Biology and Medicine, October 19, and was published in *American Medicine*, November 26.

‡ The references to the literature will be given in the paper "On the Hematozoa of Birds."

Schaudinn. So much so was this the case that our early results were interpreted at first as fully upholding his views. As the work progressed, however, it was found that the results obtained, instead of supporting, actually contradicted his conclusions.

Schaudinn's extensive memoir must be read in the original by those who are interested in the subject. The following summary will, however, serve to bring out the main points in his paper.

On allowing the common mosquito (*Culex pipiens*) to feed upon the blood of owls infected with *halteridium* (*Hæmoproteus noctuæ*) he found in the intestines of about 10 per cent of these insects large numbers of trypanosomes which he considered as stages in the life-history of this hemocytozoön. The oökinetes, resulting from the fertilization of macrogametes, showed from the start evidence of sexual differentiation. Thus, one type changed to an indifferent or asexual trypanosome, which then rapidly multiplied by longitudinal division, that is, in the same manner as do ordinary trypanosomes. Another type of oökinete gave rise to a female trypanosome which apparently was incapable of multiplying by longitudinal division, but by parthenogenesis regenerated all three kinds of trypanosomes. The third type of oökinete gave rise directly to eight male trypanosomes or microgametes, which, as such, are incapable of further development or multiplication, and merely serve to fertilize the macrogametes. This type of oökinete therefore corresponds to the free microgametocyte of the blood. All three forms of trypanosomes under unfavorable conditions agglutinate, forming rosettes with centrally directed flagella.

The indifferent trypanosomes, as a result of rapid multiplication, become very small, and when injected into the blood of an owl attach themselves by the flagellar end to the erythrocytes. The flagellum is then absorbed and the parasite appears as the well-known early stage of the *halteridium*. Usually during the night, the parasite leaves the surface of the red blood cell, at first, as a gregarine-like vermiculus which later takes on the trypanosome form. This, after a period of motion, again attaches itself to a cell and passes into a condition of rest, during which it grows until the next night when it again leaves its host. This alternation of the motile and rest periods continues until the sixth day when the *halteridium* has reached its full size, whereupon it leaves the cell and by rapid, consecutive, longitudinal division gives rise to small flagellates which then begin a new six-day cycle. The indifferent trypanosome in this way gives rise to the male and female *halteridia* of the blood.

The female trypanosomes when introduced into the blood likewise attach themselves to erythrocytes, but change their host-cells less often than do the former. When they leave the cell they do not take on the trypanosome form, but remain as gregarine-like vermiculi, which after a while enter new cells. The fully developed form appears as the typical macrogamete which no longer is able to leave the cell.

The male trypanosome or microgamete, when introduced into the blood, dies out rapidly. The male and female types result from and are constantly renewed by the differentiation of the indifferent trypanosomes or *halteridia*.

The results obtained by Schaudinn with the "leucocytozoön" of Danilewsky (*Hæmamœba Ziemanni*) were much the same as those mentioned above for the *halteridium*. When the blood of the owl which contains this parasite is sucked up by the mosquito (*Culex pipiens*) fertilization of the sexual elements occurs in the gut of the mosquito in the same way as under a cover-glass. The female cell or macrogamete ripens and becomes free; the microgametes are given off from the male cell or microgametocyte and fertilize the female cell. A large oökinete results which then grows in length — giving rise to a complicated coil. At the same time the original nucleus divides in rapid succession, and thus arise a large number of nuclei each of which becomes surrounded by a dense plasma zone. These small cell-territories, as in the case of the oökinete of the *halteridium*, develop into trypanosomes which become indifferent, male or female in type, according to the character of the original oökinete. These trypanosomes eventually swarm out of the large coils or differentiated oökinetes.

The indifferent forms have all the structure of typical trypanosomes. They multiply by longitudinal division and with such rapidity that the resulting young cells become extremely small; so small indeed as to be scarcely visible. Schaudinn expressed the belief that these small forms could readily pass through a Chamberland filter. When a cell divides, the two daughter cells remain attached by the posterior ends. Another feature to which Schaudinn calls special attention is agglutination. The resulting clumps are made up of indifferent cells which come together by their posterior ends, and hence their flagella are on the outside of the rosette, whereas in the case of the *halteridium* trypanosome the whips are directed centrally. It is because of this fact that Schaudinn designates this type as a "*spirochete*."

The female spirochetes are larger than the indifferent forms; the plasma is darker, the nucleus and blepharoplast are relatively small, and the undulating membrane is not prolonged as a free flagellum. The male spirochetes are so small as to be scarcely visible.

The sexual forms develop from the indifferent types. Owing to their rapid growth they soon become larger than their host-cells, and are therefore unable to enter these.

Such a spirochete, therefore, attaches itself by its posterior end to an erythrocyte which it then draws into itself. After it digests the plasma the nucleus is pushed off to one side as an elongated halter-shaped body, eventually to be thrown away as waste. This very novel view of the structure of the leucocytozoön is at variance with our own observations.

It is worthy of note that Schaudinn regards the spirochetes of recurrent fever and of geese as essentially the same as the preceding. There can be but little doubt that these organisms will be shown to be trypanosomes and hence unrelated to the bacteria.

It is evident from Schaudinn's work that he holds that such trypanosomes as chance to be present in the blood of birds represent not distinct species of organisms but merely stages in the development of intracellular parasites. This radical view, how-

ever, cannot be substantiated, for it will be shown that trypanosomatic infection is very common among birds, and that it bears no relation to the intracellular parasites which may or may not be present. Moreover, his two types of trypanosome and spirochete are really two forms of one species.

The reason for this difference in the conclusions arrived at by Schaudinn and ourselves lies clearly in the methods employed. There can be no question as to the fact that Schaudinn cultivated trypanosomes, *in vivo*, in the mosquito, but since this insect can be infected by several malarial parasites it follows that he really worked with *mixed cultures*, if we may thus use this term. The apparently positive results obtained by the injections of suspensions of the infected mosquitoes must be considered as due, not to the trypanosomes which chanced to be present, but to the unrecognized developmental forms of the hemocytozoa.

On the other hand, we cultivated the trypanosomes *in vitro*, under which conditions the trypanosomes multiply with great readiness, whereas the hemocytozoa die out. We thus obtained from a series of birds a large number of *pure cultures* of trypanosomes, representing several species. With such material we were unable to infect birds so as to give rise to intracellular parasites. As in the case of bacteria, the pure culture is after all the means that enables one to solve the relation of a protozoön to a certain phenomenon, which, by assumption, it may be credited to possess.

OCCURRENCE OF TRYPANOSOMES IN BIRDS.

The earliest observation on the presence of protozoa in birds is perhaps that of Gros,[34] who described organisms in the blood of the crane, crow, and fern-owl. His flagellates were 10–15 μ long, and very narrow (microgametes ?). In the crow he found a hematozoön which was 100–130 μ long, and narrower than a red blood cell. It is more likely that this was a filaria rather than a trypanosome.

Similarly, Wedl's observations[93] leave it by no means certain that he saw trypanosomes. In the fresh blood of the cherry-finch (*Loxia coccothraustes*) he found filaria and oval bodies of about the size of red blood cells. These bodies were provided at one end with a crown of cilia, and were regarded by Danilewsky as trypanomonas.

In view of the indefiniteness of these early observations it is but proper to credit Danilewsky with the discovery of bird trypanosomes. His first paper[8] on this subject appeared in 1885, and four years later a very extended description was published in book form.[12]

Danilewsky examined more than 300 birds, but gave no details as to the frequency and number of the trypanosomes found by him. They were met with in the blood of owls, rollers, lannerets, etc., and the number was said to vary with the individuals and the season. In one case they were found in young rollers (*Coracias garula*) but three or four days old. They were also found in young featherless lannerets. He observed only one form of trypanosome which to him corresponded perfectly with the *Tr. fusiforme* (*piscium*) of fish. According to the size he divided this into *Tr. majus* and *Tr. minus*. The length of the latter, not counting the flagellum, varied from 18–22 μ; while that of the former was 45–60 μ. The young forms (9–10 μ), arising by segmentation, he designated as *trypanomonas*. Whereas in the heart-blood but one or two trypanosomes could be found, and then only with difficulty, in the red marrow of bones they were detected in large numbers. It would seem as if the red marrow was the principal place where these organisms are found.

Although Danilewsky designated the trypanosomes by several names, such as *Tr. fusiforme*, *Tr. majus*, *Tr. minus*, *Tr. sanguinis avium*, *Tr. fusiforme avium*, *Tr. costatum*, it must not be assumed that these meant to him distinct species. On the contrary he held to a unity among the trypanosomes just as he did for the cytozoa of birds. Nevertheless, it is evident that he met with at least two distinct forms in respect to size. Our own results tend to confirm his view that these two forms represent stages in the development of one and the same species.

Grassi and Feletti[29] mention the presence of trypanosomes in bats, but accord no notice to those of birds, other than to express their conviction that they have nothing to do with the malarial parasites.

According to Sjöbring[88] trypanosome infection of birds is widespread about Säftstaholm in Södermannland, Sweden. He was able to find the organism in nearly all passerine birds examined by him with the exception of Corvus and Pica. The infection was apparently local, for elsewhere he did not meet with it. Beyond this mere statement he gave no detail, and merely added that the forms observed corresponded with those of Danilewsky.

Ziemann,[94] in 1898, in 190 birds examined found trypanosomes but once and that in a chaffinch (*Fringilla cœlebs*) from Heligoland. Beyond the mere mention of this fact nothing further is said. The title of a more recent paper by Ziemann,[95] which indicates the presence of a trypanosome in an owl captured in Cameroon, is misleading. As a matter of fact, he did not find a real trypanosome but, acting upon a personal communication from Schaudinn, he used this term to designate a "leucocytozoön."

According to Laveran,[55] trypanosomes are very rare in birds indigenous to France, inasmuch as he examined a large number with negative results. In an owl (*Syrnium aluco*), purchased in Paris, he found trypanosomes, but they were very scarce. This bird had a quadruple infection: filaria, *H. Danilewskyi*, *H. Ziemanni*, and Trypanosoma. The latter, whip included, measured 33–45 μ. Following Danilewsky, Laveran designated this species as *Tr. avium* and carefully described its characteristics.

Dutton and Todd,[25] on their expedition to Senegambia, were able to find a larger number of trypanosomatic infections than any of their predecessors. Of 40 birds examined eight were found to have trypanosomes in their blood.

At Bathurst, 25 birds (mostly *Estrelda* and *Crithagra*) were examined and of these only one was infected. The scarcity of the parasites in the blood may be seen from the fact that they found but from two to four trypanosomes in a cover-glass preparation. This particular species they designated as *Tr. Johnstoni*. It had a very active spirillum-like motion, but possessed no free flagellum, and the undulating membrane was scarcely recognizable. The body was long, straight, and pointed at both ends. It measured 36–38 μ in length, and 1.4–1.6 μ in width. Two larks were inoculated with the blood from this bird, but with negative result.

At St. Louis, Senegal, they examined 15 birds (*Estrelda* and *Crithagra*) and found seven of these to be infected. The species in this case was left undetermined. They were not present in large numbers, were stumpy, sluggish, and of great width. The body measured 21.6 by 8 μ; the length of the flagellum was 10–12 μ. Two pigeons and two larks were inoculated with negative results.

Hanna,[35] while in India in 1900, examined the blood of domestic pigeons which were infected with *H. Danilewskyi*, and found trypanosomes. The percentage of infected birds was not large, and the parasites were comparatively few in number. The health of the birds was not affected. The trypanosome described and figured by Hanna is 45–60 μ in length, and 6–8 μ in width. A feature which distinguishes this trypanosome from that of Laveran is the transverse position of the nucleus and the nearness to this of the blepharoplast. In this respect it agrees, it may be added parenthetically, with one of the forms obtained by ourselves.

In this same paper Hanna gives a description of some trypanosomes present in preparations made from the blood of crows by Ross, while in India, in 1898. Apparently the two forms represent different species as can be seen from the following measurements:

	Pigeon	Crow
Length of organism	45–60 μ	40–56 μ
Breadth opposite to nucleus	6–8 μ	3–4.8 μ
Length from centrosome to posterior end	19–22 μ	8–9.5 μ

In this connection it may be stated that Laveran and Mesnil[57] cite a personal communication from Donovan, who found trypanosomes at Madras in the blood of an owl (*Athene brama*).

In 1903 Edm. and Et. Sergent[86] examined 307 birds in Algeria. It is worthy of note that all but 18 harbored one or more parasites. Thus, 37 had *Hæmamœba relicta*, 155 had *H. Danilewskyi*, two had *H. Ziemanni*, 42 had filaria, and six had trypanosomes. The latter organisms were extremely rare, and were found only in the fresh blood, not in the stained preparations. We have found this to be usually the case in our own examinations. The trypanosomes were present in one out of 46 goldfinches (*Fringilla carduelis*), in two out of five black-caps (*Sylvia atricapilla*), and in three out of 10 swallows (*Hirundo*) examined. No detailed descriptions or measurements are given.

Early in 1904 Schaudinn[85] reported his studies upon the owl (*Athene noctua*), in which he found proteosoma, halteridium, and *H. Ziemanni*. The last two organisms, for reasons already given, he designated as *Trypanosoma*

noctuæ and *Spirochæta Ziemanni* respectively. By inference, rather than from actual statement, it is clear that trypanosomes were present in the naturally infected birds.

This belief is substantiated by the recent work (July, 1904) of the Sergents[87] upon owls in Algeria. They were able this time to find trypanosomes, in very small numbers, in stained preparations, and confirmed Schaudinn's observations with reference to the presence of trypanosomes in the digestive canal of mosquitoes which sucked the blood of this owl.

The presence of trypanosomes in the stomach of mosquitoes was observed prior to the work of Schaudinn and of the Sergents. Thus, Chatterjee[6] is quoted by Rogers[76] as having found trypanosomes in the anopheles near Calcutta. Not having access to the original paper we are unable to state whether the origin of this infection was established. Another illustration on this point is that given by Durham[24] in his report of the yellow fever expedition to Parà. Some specimens of *Stegomyia fasciata* were placed in a cage with a bat, and when examined later showed trypanosomes. It is probable that these came from the blood of the bat which, however, had not been previously examined. The presence of trypanosomes in bats has been noted by Grassi,[29] Dionisi,[21] Testi,[89] and by Donovan (cited by Laveran and Mesnil[57]).

Finally, to this list of observations bearing upon the presence of trypanosomes in the blood of birds must be added the recent discovery (May, 1904) by Levaditi of a trypanosome in the blood of a Java sparrow (padda or rice-bird, *Padda oryzivora*) purchased in Paris. The trypanosome is evidently rare in these birds, for Laveran[52] examined, at one time, a large number without finding any other parasite than *H. Danilewskyi*. This trypanosome has been designated by Laveran and Mesnil[57] as *Tr. paddæ*, who have also given a very full description of this new organism. Thiroux[90] working under their direction has studied this trypanosome and the results have been recently published. From the illustrations and description given by him we are inclined to consider this as probably identical with the large form of Danilewsky, the trypanosome of Hanna, and the large type found by us. The question of identity, however, can not be settled owing to the absence of a comparison of the cultures of these organisms.

METHODS EMPLOYED.

Before discussing the results obtained in this investigation it may be well to give in detail the methods which we have employed. These, in general, cover the direct detection of trypanosomes in the blood, their cultivation, and the inoculation of birds with such pure cultures.

The necessary drop of blood for the direct examination was usually obtained from the marginal vein on the inner side of the wing. For this purpose the feathers were first removed, and the skin was then washed with a little water. By means of a pair of sharp-pointed scissors the small vein was cut, and a drop or two of blood was thus obtained. The resulting injury is so slight that the smallest bird, such as a sparrow or canary, can be examined every few days during a long period of time. The selection of a small vessel gives just enough blood for the examination, and obviates the excessive bleeding which is likely to occur when a larger blood-vessel is cut.

In the case of the dead bird, the blood was taken directly from the heart, either by means of a fine Pasteur pipette, or with the aid of a platinum wire, or by direct contact of the cut surface with the cover-glass.

When examining the fresh blood under the cover-glass care was taken to apply just enough pressure so as to obtain a single layer of blood corpuscles. When this is properly done it becomes a very easy matter to detect intra-cellular parasites, even when these are present in very small numbers. This is especially true of the parasites of the red blood cells which are easily recognizable by their hyaline bodies, the "pseudo-vacuolae" of Danilewsky, and by the presence of the blood pigment or melanin. The so-called "leucocytozoa" are less easily recognized because of their resemblance to white blood cells, and the almost total absence of pigment granules. This is particularly true when their number is very small. The formation of microgametes, especially during the morning hours, is very prompt, and may attract attention where otherwise the parasite would be unnoticed.

The detection of trypanosomes in the fresh blood is by no means an easy matter, owing to their extreme scarcity. And yet this method is, as a rule, more delicate than the examination of stained preparations. When, for instance, there is but a single trypanosome in the blood under the cover-glass, it is more likely to attract attention by its movements and the agitation of the blood corpuscles than is the stained preparation in which the organism is, likely as not, concealed by a mass of cells. We have repeatedly been unable to find trypanosomes in stained specimens of blood the direct examination of which had readily revealed their presence.

This statement holds true also for the very small free hemogregarines or vermiculi. Notwithstanding their small size, their peculiar motion, and that of the blood cells with which they come into contact, as well as their characteristic refractile appearance, assists materially in their detection.

The fresh blood preparation should be examined first for the presence of filaria with a low power, such as a No. 3 Leitz objective. These forms, owing to their large size, can be readily detected thus, even when but one or two chance to be present in the specimen. The No. 7 objective is sufficient to show the other organisms, and is especially useful for rapid orientation. Of course, the oil-immersion must be used to establish the nature of doubtful parasites, and to bring out further details.

The method of staining which we used in the early part of our work was essentially that of Romanowsky as modified by Nocht. The solutions employed were:

1. An aqueous one per cent solution of eosin (Höchst).

2. An aqueous one per cent solution of medicinal pure methylene blue (Höchst).

The latter must be "ripened" before use. This is accomplished by adding one-half per cent of crystallized sodium carbonate, and allowing the solution to stand for several days in a paraffin bath at about 60°. A better and more rapid procedure is to place the flask containing the solution in a boiling water-bath for about half an hour, and during this time to pass through the liquid a stream of steam. The latter is generated in a flask from which it is carried into the solution through a drawn-out piece of glass

tubing. To this product one-half volume of one per cent methylene blue solution is then added.

The actual staining mixture is made by adding to about 10 c.c. of distilled water in an Esmarch or Petri dish three or four drops of the eosin solution. By slight stirring the eosin is distributed through the liquid after which about from two-thirds to one c.c. of the "ripened" or polychrome methylene blue solution is then added, and the whole stirred.

The blood smears, either on cover-glasses or on glass slides, are first fixed by immersion for about 10 minutes in a mixture of equal parts of absolute alcohol and ether. The cover-glasses are then floated on the staining mixture with the specimen side down. When slides are used these are also turned with the specimen side down, but with one end resting on the edge of the tilted Esmarch or Petri dish.

The specimens are allowed to remain in contact with the dye for about 15–20 minutes after which they are rinsed in tap-water and dipped for a few moments in an eosin solution of about one-half of one per cent strength. This serves to remove the excess of methylene blue, and imparts the desired contrast tint of eosin. The preparation is then rinsed and examined in water, after which, if satisfactory, it may be floated off, dried, and mounted in Canada balsam. When properly prepared the specimens will retain their color for several years.

The formation of a dirty deposit on the specimen can be avoided by the use of absolutely clean cover-glasses and by the use of a fresh eosin solution. The latter is prone to deterioration, and hence should be freshly prepared, every week or two. The preparation which is properly stained will show, in the case of bird's blood, the nuclei of the erythrocytes stained a deep red, while the remainder of the cell has a slight eosin tinge. The nuclei of the large leucocytes are stained pink-red while the plasma is blue. The blood plates which are scattered through the specimen appear as irregular discs of a bright red color. The nuclei of the parasites are stained a more or less deep red, while the plasma takes on a blue of variable intensity. It is least stained in the case of proteosoma, slightly more with halteridium, and most strongly with the larger "leucocytozoa." In the case of the latter the plasma of the male cell is stained a pale blue, whereas that of the female cell is usually stained a very deep blue.

The bird trypanosomes do not apparently stain as readily as do those of the rat and of Nagana, for it is the exception to find specimens with well-stained nuclei and flagella. More often the space corresponding to the nucleus is colorless or nearly so. The flagella are usually very indistinct, but nevertheless their presence can often be demonstrated. When the stained preparation fails to show free flagella it must not be assumed that these are absent, for an examination of the fresh living parasite, as found in the blood and especially as present in the culture, will show their presence. At no time have we found trypanosomes with the terminal, free flagella absent.

The staining of cultures is even more difficult than of the blood preparations. Not only is it difficult to stain the nuclei and flagella, but the precipitation of dye upon the cover-glass is such as to make the specimen worthless. It is only recently that with the aid of Mr. Torrey we have been able to modify

the method of staining so as to obtain satisfactory preparations of this kind. The slides thus prepared have made it possible to secure the excellent photographic reproductions of the cultural trypanosomes shown in the accompanying plates.

The cultivation method, as will be shown later, is far superior to the direct examination, inasmuch as it enables the isolation of trypanosomes when apparently none can be detected by the microscope. The method employed is essentially the same as that which we have used in our work on *Tr. Lewisi*,[64] *Tr. Brucei*,[65] [66] and *Tr. Evansi*.[70] In one respect, however, it has received an important modification. It will be remembered that in the work on *Tr. Brucei* only eight per cent of the infected animals gave cultures of this organism. In endeavoring to ascertain the reason for the failure in this large percentage of cases we found that the concentration of the meat extract, as used for the preparation of the agar, was a most important factor. The result of this inquiry has been published by one of us (Dr. MacNeal[60]). The main fact established is that an excess of meat extract inhibits, possibly by overstimulation, the development of the initial culture, whereas a smaller amount favors the growth. This is particularly true of the initial or first generation, but after this is once obtained the organism readily adapts itself to the ordinary medium.

Consequently, we have used in this work an agar prepared according to the following formula:

Extractives of 125 g. of rabbit or beef meat in 1,000 c.c. of distilled water; two per cent Witte's pepton; 0.5 per cent salt; two per cent agar; and 10 c.c. normal sodium carbonate solution.

The agar thus prepared is tubed and sterilized in an autoclave at 110° for 30 minutes. When cooled to about 50°, two volumes of defibrinated rabbit's blood are added, and the mixture is then allowed to solidify in an inclined position. When firmly set it is placed upright for a few minutes until a few drops of water of condensation appear. This liquid is then inoculated with a drop of blood taken from the heart of the bird by means of a drawn-out tube pipette. In some instances, as when large birds are used, the blood may be drawn from the median vein by means of a sterile syringe. The cotton plug of the tube is then cut off short, moistened with mercuric chloride, and the tube is covered with a rubber cap, after which, it is placed at 25° for about a week.

Under these conditions the bird trypanosomes grow readily and even luxuriantly. As already stated the cultures often succeed when there is no microscopical evidence of the presence of the flagellates. This fact in itself shows how easily they adapt themselves to the new medium. It may be safely said that their cultivation is as easy as that of *Tr. Lewisi*. As a rule it is possible to recognize the presence of growing trypanosomes in the tubes on the third day. On the sixth to the seventh day they are usually extremely abundant and very actively motile. The picture presented by such an active vigorous culture is interesting in the extreme, and varies considerably with the species of trypanosome under observation.

It will be seen that the cultivation method not only serves the purpose of demonstrating the presence of trypanosomes, but also furnishes in many cases

a positive means of differentiating species. This in itself makes it an invaluable addition to the purely morphological method of studying these protozoa. We have heretofore shown that the cultural characteristics of the Nagana, Surra, and rat trypanosomes are very different, and permit of their ready identification. It may be added that the bird trypanosomes, in culture, differ markedly from each of these three organisms as well as among themselves.

When cultivation is attempted it is always advisable to inoculate three or four tubes of the medium for the reason that it may happen that but one out of a set of such tubes may develop. This is readily understood when it is borne in mind that the number of trypanosomes may be very small, scarcely more than one in a drop.

As a routine we have found the following procedure useful: The blood is taken from the heart by means of a sterile pipette and transferred to four tubes of blood agar (one to eight meat extract), which are then capped and set aside at 25°. The remaining blood is used to make cover-glass or slide smears which when air-dried are fixed in a mixture of equal parts of alcohol and ether, after which they are stained by the Romanowsky method. After spreading the films a slide of the fresh blood is examined for hematozoa, first with the No. 3 Leitz objective, then with a No. 7, and finally, if necessary, with the oil-immersion lens. As a rule, this examination was made without the use of a movable stage. However, when examining stained and mounted preparations, it is advisable to make use of one, and for this purpose the new Zeiss model is to be recommended. This, in addition to being detachable, enables one to locate a given field, even when the slide is transferred to another microscope. Although not intended to be used with the microphotographic stand, we have so used it to great advantage.

The injection of the trypanosome cultures can be made either subcutaneously or into the breast muscle. The intraperitoneal injection we found to be rather dangerous, and for that reason we abandoned it in favor of intrapleural injections. These can be made with the greatest of ease and with the least possible injury to the bird. For this purpose the needle of the syringe is inserted obliquely through the furcular angle into the right pleural cavity. Relatively large doses, even one-half c.c., can thus be introduced into a small bird, such as a sparrow or canary. The feathers over the wish bone should be removed and the skin washed previous to making the injection.

UNSUCCESSFUL CULTIVATION EXPERIMENTS.

The immediate object of this investigation was to establish, if possible, the correctness of Schaudinn's views as to the trypanosome stages of the intracellular parasites. For this reason cultures were made, as a rule, from such birds as contained hemocytozoa. In some instances the cultures were made from birds which contained no recognizable parasites. In the case of the infected birds special care was taken to use those which were very rich in fully developed hemocytozoa, that is to say those which

gave rise to an abundance of microgametes. Nevertheless, such attempts were often fruitless, as will be seen from the synopsis which follows.

It may be stated in advance that of the tabulated 26 negative attempts at obtaining cultures (including those of three canaries), 19 were made with birds very rich in one or more intracellular parasites, such as *proteosoma*, *halteridium*, *H. Rouxii* with free hemogregarines, and *H. MacCallumi*. The remaining six birds had received previous injections of cultures of trypanosomes or other parasites. The failure in such indicates that, in the bird used, the trypanosomes, in the interval which elapsed, had disappeared from the blood. An instance of this kind was afforded by robin No. 270, in the blood of which trypanosomes were found 19 days before the culture was attempted. Only one tube was inoculated, and it failed to give a growth, showing that either the number of parasites had greatly decreased or had entirely disappeared in the interval.

Cultures were also attempted in the case of 10 robins which had a more or less intense infection with one or more of the following: *Pl. Vaughani*, *H. majoris*, *halteridium*, and *filaria*. Owing, however, to contamination with bacteria failure resulted. It is quite probable, judging by the large number of successful cultures obtained from robins, that some of these would have shown trypanosomes.

1. Sparrow, No. 53.—Received an injection of blood and internal organs of robin No. 51 which had numerous *halteridia*, a few *H. majoris*, and a few trypanosomes; died next day. Four tubes were inoculated; result, negative.

2. Sparrow, No. 76.—Received an injection of suspension of blood and organs of wren, No. 86, rich in trypanosomes. Two days later a few *proteosoma* appeared, these increased greatly, and on the fifteenth day, when very rich, it was etherized and four tubes inoculated. Result, negative.

3. Sparrow, No. 75.—Received same injection as preceding; repeated examinations negative. Twenty days later received injection of blood of robin No. 271 rich in *halteridium*, *H. majoris*, *Pl. Vaughani*. No infection; died 10 days after second injection. One tube inoculated, negative.

4. Sparrow, No. 108.—Received injection of blood of hemogregarine sparrow, No. 111. Four days later *proteosoma* were rich. Was etherized on the fifth day and four tubes inoculated; result, negative.

5. Sparrow, No. 113.—This sparrow was rich in *H. Rouxii* and in the long, free hemogregarines. Was etherized and two tubes inoculated; result, negative.

6. Sparrow, No. 123.—Extremely rich in *proteosoma*. Was etherized and two cultures made; result, negative.

7. Sparrow, No. 129.—Was like the preceding; two tubes inoculated with the heart-blood were negative.

8. Sparrow, No. 140.—Was inoculated with blood of *proteosoma* sparrow. Eleven days later, when extremely rich in segmenting forms, was etherized and four tubes inoculated. Result, negative.

9. Sparrow, No. 149.—Sparrow rich in *halteridium*. Was etherized and two tubes inoculated; result, negative.

10. Sparrow, No. 151.—Received an injection of blood of above *halteridium* sparrow. Eleven days later showed few *halteridia* and many *proteosoma*. Was etherized and two tubes inoculated. Result, negative.

11. Sparrow, No. 180.—Was inoculated with blood of *halteridium* sparrow, No, 149. No infection; died nine days later. One tube inoculated with negative result.

12. Sparrow, No. 181.—Was inoculated the same as preceding, and died also on the ninth day. No infection. One tube was inoculated with negative result.

13. Sparrow, No. 216.—Was extremely rich in all stages of *proteosoma*. Was chloroformed, and four tubes were inoculated. Result, negative.

14. Sparrow, No. 233.—Rich in *proteosoma* with few *halteridia;* was chloroformed and two tubes inoculated. Result, negative.

15. Sparrow, No, 234.—Was rich in *proteosoma* and *halteridia*. Immediately after death four tubes were inoculated with negative result.

16. Sparrow, No. 297.—Was very rich in *proteosoma*. Immediately after death two tubes were inoculated, but with negative result.

17. Sparrow, No. 307.—Was very rich in *halteridia* and in hemogregarines. Was chloroformed and one tube inoculated with negative result.

18. Sparrow, No. 313. Very rich in *proteosoma;* had some free gregarines; was chloroformed and two tubes inoculated. Result, negative.

19. Sparrow, No. 319.—Rich in all forms of *proteosoma* and in *halteridia;* was chloroformed and two tubes inoculated with negative result.

20. Sparrow, No. 338.—Was injected with a citrated suspension of cultures of strains A, B, C, D, E. Seven days later segmenting forms of *proteosoma* were found (latent infection). Died on the fourteenth day. One tube was inoculated with negative result.

21. Sparrow, No. 339.—Injected the same as the preceding; also died on the eleventh day. No infection. One tube was inoculated with negative result.

22. Chipping sparrow, No. 263.—*Halteridia* very abundant. Immediately after death three cultures were made. Result, negative.

23. Mourning dove, No. 85. *H. MacCallumi* numerous. Blood drawn from vein with syringe. Four tubes were inoculated. Result, negative.

24. Canary, No. 1.—Received an injection of trypanosome cultures, strains J. K. L. Four days later was killed by accident. Two tubes were inoculated at once, but with negative result. No infection.

25. Canary, No. 2.—Received the same injection of trypanosome cultures as sparrow No. 20 above. No infection. Was killed sixteen days later and one tube inoculated. Result, negative.

26. Canary, No. 8.— Was injected with *proteosoma* blood of sparrow. Died ten days later with *proteosoma* very abundant. Three tubes were inoculated with negative result.

The failure to obtain cultures of trypanosomes from birds richly infected with hemocytozoa may be taken to show that these two classes of parasites are entirely distinct. This conclusion will be strengthened by other observations.

RECOGNIZED CASES OF TRYPANOSOMATIC INFECTION.

The following table is a summary of the findings in birds having trypanosomes. These it will be seen comprise 15 species and 38 cases. The second column indicates the result of the microscopical examination of the blood in the fresh condition. A negative result is shown by the sign —. The third column gives the findings in stained preparations of the blood. An ordinary examination of such stains was very often negative and for that reason the slides were examined, field by field, with the aid of a movable stage. In some instances the blood was spread on the slide and such smears were covered with a glass slip 21 × 42 mm. Usually, however, cover-glasses were employed which were about 21 mm. wide. In several cases (as in S and P), three or four slide smears were examined before the one trypanosome was found. Obviously, the detection of trypanosomes in the blood when present in such small numbers is largely a matter of chance.

The fourth column shows the results obtained by the cultivation method. As will be seen at a glance this procedure offers the surest means of detecting the trypanosomes. In only one instance did the method apparently fail, and that was in the case of robin, No. 270, in which the parasite was seen 19 days before the culture was attempted. The dotted sign (.....) in this column means that cultivation was not tried. The letters designate the strains isolated, and the numbers the generations or sub-cultures through which each was carried.

The fifth column gives the name of the species found, based upon the characteristics given later on. A number of these are open to question since the cultural or other characteristics are somewhat different from the type species.

TABLE I.

SHOWING RESULTS OBTAINED WITH BIRDS HAVING TRYPANOSOMES.

		TRYPANOSOMES DETECTED IN			SPECIES		OTHER PARASITES PRESENT
		Direct	Stain	Cult're			
1. Blackbird, red-winged (*Agelaius phœniceus*)	No. 401	−	+ 1 small, 2 large	.....	*Tr. avium*		Filaria only.
2. " rusty (*Scolephagus carolinus*)	No. 420	−	−	A′ 10	Tr. sp.?	Type 4	None.
3. Bluebird (*Sialia sialis*)	No. 426	−	−	B′ 8	*Tr. avium*		Filaria only.
4. Blue jay (*Cyanocitta cristata*)	No. 244	+	+ 8 small, 18 large	K 10	" "		*Hœmoproteus majoris.*
5. " " "	No. 273	−	−	M 2	Tr. sp.?	Type 4	None.
6. " " "	No. 278	−	+ 18 small, 4 large	R 12	*Tr. avium*		*H. Danilewskyi*, very rich, very few *H. majoris.*
7. " " "	No. 408	−	−	Z 9	" " ?	Type 1*a*	Filaria only.
8. " " "	No. 429	−	−	C′ 9	Tr. sp.?	Type 4	*H. Rouxi?* Gregarines?
9. Dove, mourning (*Zenaidura macroura*)	No. 5	+	+ 5 small	A 7	*Tr. avium*?	Type 1*a*	*H. Sacharovi*, very abundant.
10. " " " "	No. 6	+	+ 1 "	B 22	" " ?	Type 1*a*	" " " " few.
11. Gold-finch, American (*Spinus tristis*)	No. 353	−	+ 1 "	S 10	*Tr. Laverani*		" few; *H. Danilewskyi*, rich.
12. " " " " "	No. 354	−	−	X 9	Tr. sp.?	Type 4*a*	None.
13. Flicker (*Colaptus auratus*)	No. 388	−	−	U 10	*Tr. avium*?	Type 1*a*	None.
14. Hawk, red shouldered (*Buteo lineatus*)	No. 350	−	+ 1 large	P 11	*Tr. Mesnili*		*H. Ziemanni*, *H. Danilewskyi.*
15. Oriole, Baltimore (*Icterus galbula*)	No. 41	+	+ 92 small	C 5	*Tr. avium*		None. *H. Rouxi?*
16. " " " "	No. 272	+	+ 8 "	.....	" "		None.
17. Robin (*Merula migratoria*)	No. 46	−	−	AB 4	" "		*H. majoris*, very few.
18. " " "	No. 50	−	+ 2 small	G 4	" "		*Pl. Vaughani*; few *H. Danilewskyi.*
19. " " "	No. 51	+	+ 9 "	D 5	" "		*H. majoris*, rich in *H. Dan'l'skyi.*
20. " " "	No. 53	+	+ 1 "	E 4	" "		" " " " "
21. " " "	No. 54	−	+ 9 "	F 1	" "		" " " " "
22. " " "	No. 184	−	−	I 2	" "		" " few.
23. " " "	No. 214	+ 3 large	−	L 2	" "		" " "
24. " " "	No. 242	+ 2 large	−	J 2	" "		" " "
25. " " "	No. 257	−	−	Q 12	" "		" " "
26. " " "	No. 270	+	+ 1 small, 1 large	− ?	" "		" " " Culture attempted 19 days after tr. were seen.
27. " " "	No. 284	−	−	N 2	" "		*H. Danilewskyi*, few.
28. " " "	No. 345	−	−	O 1	" "		None.
29. " " "	No. 376	−	−	V 12	" "		*H. Danilewskyi*, very few; filaria.
30. " " "	No. 393	−	−	W 9	" "		" " *Pl. Vaughani.*
31. Sparrow, English (*Passer domesticus*)	No. 109	+ 1 large	−	.....	" "		None.
32. " " " "	No. 279	+ 2 large	−	.....	" "		None.
33. " " " "	No. 286	+ 1 large	−	.....	" "		None.
34. Song sparrow (*Melospiza fasciata*)	No. 142	−	+ 2	H 4	" "		*Pl. relictum*, very rich.
35. " " " "	No. 288	+	+ 28 small, 1 large	.....	" "		*H. Danilewskyi*, rich.
36. Brown thrasher (*Harporhynchus rufus*)	No. 386	+	−	T 8	Tr. sp.?	Type 3*a*	Filaria.
37. Woodpecker, hairy (*Dryobates villosus*)	No. 391	−	+	.....	Tr. sp.?		None.
38. House wren (*Troglodytes aedon*)	No. 86	+10	−	.....	Tr. sp.?		None.
Microscopical detection failed entirely in 14; these recognized by culture		16	17	29			15 without intracellular parasites

The last column gives a list of the other parasites present in each bird. Several of the names are of new species which will be described in the next paper.

The above table embraces the results obtained from an examination of 431 birds, representing 40 species. Of this number 38 (16 species) or 8.8 per cent were found to contain trypanosomes. This figure must not be taken to indicate the actual number of birds infected with these organisms for the reason that cultures were not attempted in all cases.

The actual number of cultivation experiments (free from contamination) made with wild birds, as seen from this table and the preceding summary, is only 53. Twenty-four of these were negative and 29 were positive. Of the cultural attempts, then, 55 per cent were successful. In eight other birds trypanosomes were found with the microscope, and, if at the time an attempt at cultivation had been made, judging from the success under like conditions, the cultures would probably have developed, in which case 37 out of 61 would have been positive, or about 60 per cent.

This statement may be somewhat misleading, for the reason that of the 61 birds in question, 18 were known, from the microscopical examination made at the time, to have trypanosomes. If, therefore, these are deducted, it will be seen that in the 43 birds in which the original microscopical examination failed to show trypanosomes the cultural method revealed their presence 19 times, or in about 44 per cent.

It is evident from these considerations that the percentage of birds infected with trypanosomes is much higher than would be indicated by the limited findings given in the table. It is probable that the careful application of the cultural method to a large series of birds will show that fully one-third, if not more, harbor these parasites.

It is certainly remarkable that the microscope, even after most careful re-examination, should show trypanosomes in only 24 out of 431 birds, whereas the cultural method applied in only 53 cases, excluding attempts in which contamination occurred, should give 29 positive results.

The relation of the intracellular parasites to the trypanosomes

is of especial interest. In connection with the summary of the unsuccessful cultivation trials it was pointed out that, although cytozoa were very abundant in 19 of those cases, yet no culture was obtained. By contrast the above table shows that trypanosomes may be present, at times in very appreciable numbers, unaccompanied by intracellular parasites. Cytozoa were absent in 15 and present in 23 of the birds known to have trypanosomes.

The existence of a latent infection with cytozoa in these 15 cases is possible, but even with that assumption it is difficult to reconcile this finding with the supposed relation of trypanosomes to this class of organisms. Moreover, it can be shown that birds may harbor trypanosomes for weeks and months without showing any infection with intracellular parasites.

In one instance, in particular, a canary developed trypanosomes in its blood four days after an injection of a culture of strain D. The trypanosomes were found at times, though not always, during the following 11 weeks, when the bird died, but at no time was there any indication of the presence of a cytozoön.

Thiroux,[90] the only one previous to ourselves who has succeeded in infecting birds with trypanosomes, found that the period of incubation ranged from a few (12) hours to 18 days. This variation was due, in part, to the natural resistance of the bird (padda), and, in part, to the method of infection. The inoculations were subcutaneous, intramuscular, intravenous, or intraperitoneal; the latter being the surest method. The infection was of variable intensity, in some cases the parasites being scarce, while in others they were very numerous. Indeed, one of the padda birds died apparently from the severe infection which developed. In the case of moderate infection, usually following a short period of incubation, the trypanosomes increased in numbers during a period of 9 to 15 days after which they decreased and finally remained stationary, in some instances during a period of 40 days. In addition to the padda, five other species of birds, including the green and common canary, were successfully infected.

The significant feature of Thiroux's experiments is that, notwithstanding this rich experience in the artificial infection of birds with trypanosomes, no mention is made of the appearance of

intracellular parasites which it is reasonable to expect would appear if the latter were but stages in the life-history of the former. The fact that no mention is made of the presence of cytozoa leads us to believe that they were not found. With this assumption, his results are in accord with ours on the canary mentioned above. They agree furthermore with the observed fact noted in the table that trypanosomatic infection of wild birds may exist without association with intracellular parasites.

Furthermore the study of the trypanosomes found in the blood, and also of those obtained by cultivation, shows that there are several distinct species which exhibit no constant association with a given cytozoön. Thus, the most common species, described further on as *Tr. avium*, was associated with *H. Sacharovi*, *H. majoris*, *H. Danilewskyi*, *H. Rouxi*, *Pl. Vaughani*, or with *filaria* in addition to its very frequent single occurrence. Under these circumstances it would indeed be difficult to establish a relationship with any of the intracellular organisms mentioned.

MORPHOLOGY OF TRYPANOSOMES IN BLOOD.

Observations made upon the trypanosomes found in the fresh blood or in stained preparations are insufficient for the purpose of identification. As in the case of bacteria, the cultural characteristics and the animal experiment must, so far as possible, be utilized in order to differentiate allied organisms. In the study of the trypanosomes of mammals it has been, after all, the animal experiment which has served the purpose of identification, since the morphological variations are at times so slight as to be almost negligible. In three of these infections the cultural method has supplied an additional means of recognition. Without doubt, the number of species of trypanosomes met with in mammals and man is very large. Moreover, with perfected methods it will be found that a given species of mammal may be subject to natural infection with several kinds of trypanosomes, some of which may be very pathogenic, while others are not. In other words infections of this type will be found to parallel those of other protozoa and even of bacteria. It will be sufficient to take a single illustration—that of malaria. Malarial infection, as met with in man,

monkeys, bats, and birds, is due to different species of parasites. And, in the same species of animal, as man or bird, several species of these parasites may be found. That it is possible for one species of bird to harbor two kinds of trypanosomes will be shown farther on.

As indicated above, a thorough study of a given trypanosome embraces observations upon the living and stained organism as found in the blood, an examination of its cultural characteristics, and a determination of the pathogenic action of the pure cultures upon the same species as the host, as well as upon other species. It has not been possible to meet all these requirements in every case of trypanosome infection in the present investigation for reasons which will be readily seen.

In the 24 cases recognized by means of the microscope, the trypanosomes were found in the living condition in the blood only 16 times. The stained preparations showed them to be present in 17 cases. In 14 of the birds the examination of the fresh and stained blood was negative, the organisms being detected solely by the cultural method. It should perhaps be stated that the number of positive stains would have been much less than that given above were it not for the very valuable check afforded by the cultivation process. It happened several times that the ordinary examination of the stain was negative, but inasmuch as the culture eventually developed it led to a re-examination of the slides, field by field, and in a few instances the laborious search was rewarded by finding the trypanosomes in the stains. In the case of culture S (*Tr. Laverani*) only one trypanosome could be detected thus on three slides made from the original blood. Similarly, only one could be found on four slides of the blood which gave culture P (*Tr. Mesnili*).

The difficulty of detecting trypanosomes in stained preparations can best be shown in the case of a canary which was artificially infected by means of culture D. During a period of 11 weeks the parasites were repeatedly found in living condition in the blood, and, although stains were made at the same time, they were invariably negative. When the bird died trypanosomes were found as usual, but, although seven slide smears

were made and carefully examined on a movable stage, the examination consuming about 20 hours, the result was the same as before.

With but few exceptions, the trypanosomes which we found in the living state in the blood were one of two types, corresponding very closely to the *Tr. majus* and *Tr. minus* of Danilewsky, so much so that we are obliged to consider our common forms as identical with the ones described by him. Moreover, we are in accord with Danilewsky in regarding these two forms, notwithstanding their great difference in size, as belonging to one and the same species—*Tr. avium.* This statement, however, refers only to the common trypanosomes of birds since, as will be seen, there are several species.

In five of the birds, as can be seen from the table, the large and small forms were found together. At first sight, it would appear as if the two forms represented distinct species, but that such is not the case is readily seen from the cultural characteristics. In other words, the cultures made from the blood of birds having only the small form, or only the large form, or both forms at the same time, present exactly the same appearance. The position of the blepharoplast with reference to the nucleus clearly shows that the large form is to be considered as preparing for division. Furthermore, the presence of this type in very young birds indicates that the infection is of recent origin. In this respect it corresponds to the large form of *Tr. Lewisi* which appears in the blood on about the sixth to the ninth day of infection. As is well known, in the rat after the stage of active multiplication of *Tr. Lewisi* is passed, the large form disappears, and only the ordinary or typical form persists. That there is a close generic relation between *Tr. Lewisi* and *Tr. avium* is seen in the marked similarity of the cultures, both giving rise to actively motile free-swimming forms, and to characteristic multiplication rosettes, the individuals composing which have their flagella directed toward the center.

In view of these facts we are led to regard this large form as a multiplication, or possibly sexual type, of the smaller and more common form.

This large form usually appears as an S-shaped body having

a well-developed, undulating membrane, which terminates in a free flagellum 10–15 μ long. The body proper varies in length from 21–30 μ, but at times it may attain even 40 μ and more. The posterior end for about 12–15 μ is narrow and tapers to a point. It shows a peculiar stiffness, due without doubt to the fact that the blepharoplast is near the nucleus. The nucleus is readily visible in the living form as a large round body, or, more often, as an oval which is placed transversely across the body, filling out the entire width. The width of the cell opposite the nucleus is from 5–7 μ. As might be expected, owing to its large size, this form shows very little tendency to travel out of the field of the microscope, and for that reason can be readily kept under observation for hours. The contortions, however, are very active, and the organism is constantly changing from side to side, at times straightening out, or forming a coil. For that reason the measurements as given above, made on the living parasite, are only approximate.

The stained preparation shows the large form in the characteristic S-shape (Plates 2 and 3) or in the circular position (Plates 3 and 4). The centrosome, it will be seen, lies close to the nucleus and is in a large colorless space. The nucleus itself does not stain readily, and for that reason appears as a large light body in the middle of the cell. Beautiful striæ or myonema lines, six or eight in number, are easily made out on the part overlying the nucleus. These lines are continued the entire length of the cell. The long stiff or atrophied posterior end is very noticeable. The undulating membrane is visible as a delicate, fairly wide, and rather wavy border on one side of the organism. As will be seen from the table, the length of the body of the large form varies within wide limits, that is from 35–65 μ. The whip is probably 15–20 μ long.

The two forms described represent the common types as met with in the blood of birds, and are to be regarded as belonging to the same species. Two other trypanosomes, however, were found in the blood.

The first of these met with, in the blood of a hawk, is shown in Fig. 1, Plate 5. The large size (see culture P, Table 2) and peculiar shape mark it at once as distinct from the preceding.

Moreover, the cultural characteristics are totally different and stamp it as a new species. We have named it *Tr. Mesnili* in honor of Dr. Mesnil, of the Pasteur Institute. The full description of this form will be found later on.

The second trypanosome, referred to above, was found but once in the stains made from the blood of a goldfinch. It is designated as Culture S in the table, and from the measurements there given it will be seen to correspond closely to the ordinary small form of *Tr. avium.* Indeed, if the size was the only criterion, there would be little hesitation in regarding it as being of the same species. The presence of a large number of granules in the posterior half would hardly be sufficient evidence for separating it as a distinct species. And yet the cultural features of this organism are unlike those of *Tr. avium* or of *Tr. Mesnili*, and on that account it must be considered as a new species. We have designated this organism as *Tr. Laverani* in honor of Dr. Laveran.

The small form is the most common one met with in the blood of birds. The total length, whip included, is about 25–30 μ, or a trifle more than the length of two blood corpuscles. The body proper is about 20 μ long, though exceptionally it may vary from 14 to 25 μ. The width ranges from 3.5–5 μ. The nucleus, of nearly the same width as the cell, is at times visible, but not always. The posterior half of the body usually shows minute granules or globules, but at no time is there any indication of pigment granules. The body is usually in the form of a straight spindle, widest in the middle, tapering quite evenly in both directions. The anterior end is usually bent at right angles to the line of the body. There is an absence of the stiff posterior end as met with in the larger form. The undulating membrane is easily made out, though it is not as wide as in the other type. It starts very close to the posterior end, and as it passes forward, crosses the body, forming one or two wavy bends. It finally terminates in a free flagellum, 8–12 μ long. The motion is sluggish, and, as in the other form, there is but little tendency to travel out of the field of view.

The small form as seen from the photographs (Plates 1–4) is a spindle-shaped body which tapers to a sharp posterior end.

The large centrosome or blepharoplast at times appears to be at the very tip, while again it may be four or five μ distant. It is often surrounded by a colorless or achromic zone. The flagellum, in several of the preparations, is seen to start from the centrosome and form the outer border of the undulating membrane, eventually becoming the free whip, which is about 8–10 μ in length. The nucleus is not always distinct, but it can often be seen as a large body nearly as wide as the cell. In the preparations which have become crushed or flattened out (see Fig. 4, Plate 1, and Fig. 2, Plate 3) the nucleus and border of the undulating membrane can be seen very plainly. In a few instances a faint suggestion of striæ on the surface of the body can be made out.

The description and measurements of the two forms as given above, unless otherwise indicated, refer to the organisms as found in the living condition. Obviously, it is not possible on account of the constant movement to make very accurate measurements, whereas with the stained preparation this can be done very satisfactorily. The stain has the further advantage in that structural differences are readily brought out, if any exist.

The measurements given in the appended table include all the kinds of trypanosomes met with in the stained preparations. In the few instances where the number of organisms was large, only five or six were taken for this purpose.

The small forms are grouped together in order to show the close similarity in size. At first sight it would seem as if there were two types, one of which was about 20 μ in length with the centrosome close to the posterior end, usually not over 1 μ from the tip; while the other was about 25 μ in length with the centrosome about 5 μ from the end. In view of the fact, however, that the cultural features are so closely alike, if not identical, it has not seemed desirable at present to place too much stress on this slight difference, especially as a still greater variation is met with in the case of the large form which, as mentioned above, must be regarded as belonging to the same organism. It is quite probable the larger of the small forms, with its centrosome at a distance from the tip, constitutes a transition to the large form which has its centrosome close to the nucleus.

TABLE II.

GIVING MEASUREMENTS OF TRYPANOSOMES AS FOUND IN STAINS.

Trypanosomes Found in		Length of Free Flagellum	Length of Body	Distance from Centrosome to Posterior End	Distance from Centrosome to Anterior End	Distance from Centrosome to Center of Nucleus	Width of Body at Nucleus	Size of Nucleus	Length of Centrosome
Baltimore oriole, No. 41, culture C.	*a*..	8.0	21	1.0	20	10	5.0	3×5	0.7
	b..	....	20	0.5	19.5	10	5.0	4×3	0.7
	c..	....	20	1.0	19	12	5.0	5×4	0.7
	d..	....	21	0.5	20.5	10	4	4×3	0.7
Robin, No. 53, culture E.	*a*..	....	20	0.5	19.5	11	5.0	2.5×3	1.0
Robin, No. 270, No culture. Small form.	*a*..	8.0	20	0.5	19.5	12	5.0	4×4	1.0
Blue jay, No. 244, culture K.	*a*..	10	18	1.0	18	11.5	5.0	4×3	1.0
Small form.	*b*..	....	19	1.5	17.5	8.5	5.5	5×3	0.5*
	c..	....	16	1.5	14.5	9.5	5.2	5×3	0.7*
	d..	....	18	1.0	17.0	9.0	4.0	4×2	1.0
	e..	....	19	1.0	18.0	9.0	4.0	3×2.5	1.0
	f..	....	18	1.5	16.5	9.5	7.0	6×3	1.0*
Goldfinch, No. 353, culture S. A distinct species.	*a*..	....	20	1.0	18.0	8.	6.0	4×2	0.6
Song sparrow, No. 142, culture H.	*a*..	9.0	20.0	1.5	18.5	8.5	4.5	4×3	0.6
	b..	....	19.0	1.5	17.5	9.5	9.0	6×3	0.7*
Mourning dove, No. 5, culture A.	*a*..	6-8	22	1.0	21.0	10.0	5.0	4×4	1.0
	b..	....	23	1.0	22.0	10.5	5	4×5	0.7
	c..	....	22	1.0	21	9.5	4.0	3×4	0.7
	d..	8	22	1.0	21	9.0	4.5	3×4	0.5
	e..	....	24	1.0	23	10.0	4.5	4×4	0.7
Mourning dove, No. 6, culture B.	*a*..	8-10	24	1.0	23	10.0	5.0	4×4	0.7
Robin, No. 50, culture G.	*a*..	10	16	1.5	14.5	6.5	6.0	3×5	0.7*
	b..	9-10	23.5	5.0	18.5	6.5	6.0	3×5	0.5
Robin, No. 51, culture D.	*a*..	5.5+	23	4.0	19	12	5.0	4×5	0.7
	b..	9.0	25	5.0	20	10	5.0	4×5	0.7
	c..	....	25	5.0	20	10	5.0	4×5	1.0
	d..	....	25	5.0	20	10	5.0	4×5	1.0
	e..	....	24	5.0	19	9+	4.0	....	0.7
Blue jay, No. 278, culture R.	*a*..	9.0	24	6.0	18	7.0	5.0	4×4	0.5
Small form.	*b*..	13	25	5.0	20	7.0	5.0	4×5	0.5
	c..	8.0	24	5.0	19	8.0	5.5	4×5	0.5
	d..	....	24	5.0	19	7.5	5.0	3×4.5	0.5
	e..	7	24	5.5	18.5	8.5	5.0	3×5	0.5

*Indicates that the specimen was somewhat crushed or flattened. As a result it is shorter and wider than usual.

TABLE II—*Continued.*

Trypanosomes Found in		Length of Free Flagellum	Length of Body	Distance from Centrosome to Posterior End	Distance from Centrosome to Anterior End	Distance from Centrosome to Center of Nucleus	Width of Body at Nucleus	Size of Nucleus	Length of Centrosome
Robin, No. 54, culture F.	*a*..	....	20	4.0	16	8.0	5.5	2×5	1.0
	b..	8.0	21	4.0	17	7.5	5.0	3×4	1.0
	c..	....	20	3.5	16.5	8.0	5.5	3×5	0.7
	d..	....	20	4.0	16	7.5	4.0	2×3	1.0
	e..	....	22	4.0	18	8.5	5.0	3×5	1.0
Song sparrow, No. 288, No culture. Small form	*a*..	8+	18	2.0	16	7.0	5.0	4×4	1.0
	b..	....	17	4.0	13.0	6.0	4.0	3×3	0.7
	c..	....	19	3.0	15.0	10	3.5	3×3	0.5
	d..	....	17	2.0	15.0	8.0	4.0	3×3	1.0
	e..	....	20	3.0	17	8.0	4.0	3×3	0.7
	f..	....	21	1.5	19.5	10.0	5.0	4×4	1.0
Baltimore oriole, No. 272, No culture.	*a*..	10	20	4.0	16.0	7.0	5.0	2.5×2	0.5
	b..	8	20	5.0	15	8.0	6.0	2.5×3	0.5
	c..	6.0+	21	5.0	16	8.0	4.0	2×3	0.5
	d..	10	21	5.0	16	8.0	4.0	2×3	0.5
	e..	8	17	3.0	14	6.0	7.5	3×5	0.5*
	f..	7+	20	5.0	15	9.0	7.0	3×5	0.7*
	g..	7	22	6.0	16	8.0	6.0	3×5	0.7*
	h..	7	21	2.0	19	9.0	6.5	3×3	0.7
Blackbird, No. 401, No culture.	*a*..	....	35	15	20	6	4.0	3×3	1.0
	b..	....	35	15	20	6	5.0	5×5	0.7
Song sparrow, No. 288, No culture. Large form	*a*..	....	40	15.0	25	7.0	4.0	4×4	0.7
Blue jay, No. 278, culture R. Large form.	*a*..	....	53	19.0	34	7.0	5.0	4×5	0.5
	b..	20.0	50	17.5	32.5	6.5	6.5	5×5	0.5*
	c..	*Ca.*10	49	13.0	36	10	6.5	5×5	0.5
	d..	12	52	19.0	33.0	7.0	5.0	4×5	0.5
Blue jay, No. 244, culture K. Large form.	*a*..	....	50	20	30	7.0	5.0	4×5	1.0
	b..	10+	48	18	30	7.0	5.0	4×5	1.0
	c..	16.0	57	25	32	7.0	6.0	4×5	1.0
	d..	15 0	53	23	30	7.0	5.0	4×5	1.0
	e..	10–15	53	24	29	7.0	5.0	4×5	1.0
Robin, No. 270, No culture. Large form.	*a*..	....	65	20	45	7.5	5.0	5×5	1.0
Hawk, No. 350, culture P. A distinct species.	*a*..	....	50	7.0	43	11.0	8.0	2×3	1.0

*Indicates that the specimen was somewhat crushed or flattened. As a result it is shorter and wider than usual.

CULTURAL CHARACTERISTICS OF THE TRYPANOSOMES STUDIED.

Very little difficulty is experienced in cultivating the bird trypanosomes. As a rule they grow quite rapidly, so that at 25° their numbers are quite appreciable on the third day. They reach their maximum on about the seventh or eighth day after which they give rise to spherical involution forms which soon agglutinate or gather into large masses. These become coarsely granular, show highly refractive bodies, and eventually break down into a mass of granular débris. Living forms are rarely to be seen after about two weeks, although, exceptionally, we have met with them at the end of a month. When grown at room temperature, the culture naturally comes on more slowly and remains alive for a much longer period.

Of the 29 strains obtained from that number of birds, only two (strains S and T) showed a much slower growth. In these the trypanosomes were not appreciable until on about the sixth, and did not reach their maximum until about the tenth day.

In all cases the cultures when fully developed were enormously rich in the flagellates. At times there were observed on the surface of the blood agar, just above the fluid, circular colonies, two to three mm. in diameter, which were suspected to be due to bacterial contamination. An examination, however, of such colonies showed them to be a solid mass of actively wriggling trypanosomes.

Subcultures were obtained from all but two (strains F and O) which died out through oversight. In a number of instances these cultures have been kept up for over six months, during which time they have passed through more than 20 generations. Most of the strains have been kept up continuously for the past four months and continue to maintain their original characteristics. A number accidentally died out on account of delayed transplantation.

An examination of the 29 strains in the living condition shows at a glance that they comprise several distinct types, and it may be as well to speak of these for the present by that designation.

The division into types is based upon the characteristics shown by the rosettes and by the free-swimming forms. The main points in this grouping of the cultures can be summarized thus:

Type 1.—Rosettes and free forms common. The latter are very long and narrow, mere threads, without any noticeable enlargement of the body (*spirochetes*). Growth rapid. In these the blepharoplast is posterior to the nucleus. This is the most common type and corresponds to *Tr. avium.*

Sub-type 1a.—This is much like the preceding, but the spirochetes are shorter and are distinctly wider near the anterior end. They remain, however, long and narrow. Represented by strains A, B, U, and Z.

Type 2.—Large rosettes, the cells being of considerable size and having long central whips. The free forms are likewise large and wide, and show globules. Growth rapid. Represented by strain P—*Tr. Mesnili.*

Type 3.—Rosettes much less common and smaller. The free cells show relatively but little motion, have a blunt posterior end, and the contents are largely made up of big globules. Growth very slow. The cells show a posterior terminal rod. Represented by strain S—*Tr. Laverani.*

Sub-type 3a.—Growth equally slow as preceding. The free cells have a tapering posterior end. Represented by strain T.

Type 4.—Rosettes are very scarce, the free forms predominating. The anterior end of body tapers out gradually along the whip; the cell tapers also posteriorly, contains very small granules, and is actively motile. Rapid growth. Represented by strains M, A′, and C′.

Sub-type 4a.—This is represented by strain X. The rosettes are even more scarce. The growth is rapid and rich. The blepharoplast is very large.

Type 1.—This will be described as *Tr. avium* since it represents the most common form met with in birds. It was present in 18 out of the 29 cultures. In seven birds from which cultures, however, were not obtained the trypanosomes presented the characteristics of *Tr. avium.* It appears therefore that this species was present in 25 out of 38 birds. The particular birds in which this species was found are indicated in Table I.

Two forms are met with in cultures of this type: (1) rosettes of round, oval, or spindle-shaped cells; (2) extremely slender, long, wavy, darting forms. These will be described as "*spirochetes*," since they correspond exactly to what Schaudinn has designated by that name in connection with his work upon the "leucocytozoön" *H. Ziemanni.* The relative abundance of the two forms varies somewhat in the different strains. Thus, in some the rosette form is very abundant from the start, and the spirochetes are rather scarce, whereas in others the latter appear first and in large numbers, while the rosettes are few and small. Again, in some strains, the spirochetes appear to be much longer than in others. These differences we are inclined to look upon

at present as variations in the several strains rather than as indicative of separate species.

Rosettes.— (See Plates 8 and 9.) In the early stage the rosette consists of a small number of round bodies which may show a few minute granules. They are about 5 μ in diameter and show very little or no motion. They increase rapidly in numbers by means of longitudinal division, giving rise to aggregates of hundreds of cells. Frequently rosettes are met with which, in the somewhat flattened condition due to the pressure of the cover-glass, fill the entire field of a No. 7 Leitz objective.

Eventually, the round or pear-shaped bodies elongate to form ovals, and finally spindle-shaped bodies. At this stage the individuals may be seen to possess a slight swaying motion. The smaller rosettes, consisting of 10–20 cells, measure 14–20 μ in diameter. The spindle-shaped cells are about 10 μ long and about 3 μ wide. They do not apparently break loose from the group, but remain attached. When the growth has reached its full age the spindles begin to form the spherical involutions mentioned above.

It will be seen that the ordinary rosette corresponds in diameter to twice the length of the spindle-shaped cell, that is to say, about 20 μ. The very large rosettes really consist of a number of such smaller ones which can be easily made out in the living condition. The latter may be spoken of as simple or primary and the former as multiple rosettes.

Preparations stained by the Romanowsky method reveal details which cannot be satisfactorily established otherwise. In the first place, the flagella, as in the case of the rosettes of *Tr. Lewisi*, are found to be directed centrally. The relatively large blepharoplast, in an achromic area, is by the side of, or anterior to, the nucleus. This necessarily means that the undulating membrane is very short or even rudimentary, and this explains the absence of motion in the round or oval forms and the slight motion seen in the more elongated bodies. It would appear from this fact that the active motility of the free trypanosome is due largely, if not wholly, to the undulating membrane, and that the free flagellum is incapable of moving or propelling the body.

Furthermore, the stained preparation throws some light upon the formation of the rosette. It appears that they originate in much the same way as the segmentation form of *Tr. Lewisi* in the blood, described by MacNeal[61] and others. In very young cultures it can be seen that the large, free, spindle-shaped trypanosome shortens to form an oval or pear-shaped body, while at the same time the whip is retracted or absorbed (See Plate 9.) The blepharoplast and the nucleus then divide, giving rise to a pear-shaped body with two nuclei and two blepharoplasts. The cell itself may then divide longitudinally, forming two small cells; or, the division of the nuclei and blepharoplasts may be repeated, resulting in a cell with four each of these bodies. In the case where two cells form by the division of the original, they remain attached for some reason by their rudimentary whips, and, as the process of division is repeated, eventually the typical rosette forms. These groups therefore originate by the consecutive division *in situ* of a single cell, and for that reason the multiplication rosette must not be confounded with agglutination masses. The young forms resulting from the division of the oval cell, especially when the consecutive division is rapid, are quite small, about 5 μ long and $2\frac{1}{2}$ μ wide.

The content of the spindle-shaped cells comprising the rosette stains a deeper blue than does that of the spirochete form. The nucleus is always round, large, and shows eight chromosomes. At times a few small colorless granules and globules are present in the cell. These, however, are insignificant in size when compared with similar bodies found in *Tr. Brucei*, *Tr. Mesnili*, and the other types mentioned later.

Spirochetes.—(See Plates 10 and 11.) These are extremely interesting on account of their very rapid motion and their delicate appearance. As seen in the living preparation they appear as long, slender threads, straight or wavy in outline. They are ordinarily single and dart through the field at great speed. Usually, a very fine whip may be seen at one end, but, at times, this may be so delicate as to be invisible. They frequently attach themselves by means of their whip to red blood cells, which are then rapidly pushed through the field. The spiro-

chete, as it travels with the whip foremost, may suddenly stop and move backwards for a short distance.

Dividing forms in the young culture can be observed. The division takes place while the cell is in active motion and without any shortening or rounding up as in the case of the rosette type. The stained preparations are especially useful to bring out this condition. The two young cells resulting from division may remain attached for some time at the posterior end, and, in that case, may simulate an agglutinated pair. The two cells, however, are not in a straight line, but form an acute angle, or they may twist about each other in a spiral manner. Each of the cells, while still attached, may undergo division, thus giving rise to a four-celled group.

The spirochetes show a marked tendency to agglutinate. This begins with two cells sticking together at their posterior ends which slightly overlap. (See Figs. 3 and 4, Plate 10.) In the living preparation the line of junction can not be made out, and as a result the double cell appears to be a single organism with one whip at each end.

A third cell may attach itself by its posterior end to the agglutinated pair, in which case the three bodies may be fairly equidistant. Finally more cells come in and join in the same way, eventually giving rise to tangles of hundreds and even thousands of cells (Fig. 5, Plate 11). We have observed masses of these slender, writhing forms fill the entire field of a No. 7 objective. In these groups or masses the whips are always on the outside or periphery, whereas in the rosettes described above the whips are always on the inside.

It will be seen that the two kinds of groups met with in cultures of this organism are of entirely different origin. The multiplication rosette, with its whips directed centrally, arises by the consecutive division of its cells *in situ*, and is in nowise to be looked upon as an agglutination. On the other hand the spirochetes are normally free and divide while in that condition. These free cells agglutinate in some cultures more readily than in others, in which case the cells join at their posterior ends. The very large rosette always shows the constituent groups of

which it is composed, whereas the agglutinated mass of spirochetes, is without any such regularity.

As the cultures age the spirochetes lose their even form and show a globular enlargement at the posterior end or near the middle. This spherulation may continue until the long form is replaced by a small round body which usually is indicative of the death of the cell. Death may, however, occur without this transformation being completed.

The width of the spirochete in the living condition is about 0.5 μ. The stained preparation, perhaps as a result of flattening, shows the width to be about 1.0 μ. The average length of the spirochete is about 30 μ, but it is not uncommon to meet with cells 50 and even 60 μ long (Plate 10, Fig. 2). The body tapers at each end to a sharp point.

The stain shows that the free flagellum is relatively very short since it measures only about six μ. The blepharoplast, which stains more readily than either the nucleus or whip, is a small dot about 0.5 μ in size. Unlike that of the spindles of the rosette form, it is situated between the nucleus and the posterior end, and distant from the latter by about one-third the length of the body. That is to say, with the body length of 30 μ the blepharoplast is 10 μ from the posterior end. Consequently, the undulating membrane extends along two-thirds the length of this slender body. This explains the extreme motility of this type. Moreover, the shortness of the free whip indicates that it has very little to do with the motion of the cell, as has been pointed out in connection with the rosette form. The position of the blepharoplast, posterior to the nucleus, is an important characteristic, and is not seen in the other species.

The nucleus lies in the anterior half of the body and is approximately of the same width as the cell. In the average spirochete it measures 1×3 μ, while in the very long ones it may be 1×5 μ. When the cell is about to divide the blepharoplast approaches and almost touches the nucleus. At the same time the nucleus becomes round and the cell widens somewhat.

The contents of the spirochete are usually perfectly homogeneous, and in the stains only a few minute granules can be made out

Owing to the marked difference between the rosette and spirochete forms it may be supposed that they represent two distinct species. This view, however, is untenable since these two forms are present in every one of the 18 strains. Moreover, at no time have we found a culture which had either form by itself, as could well be expected, if they were distinct species. Again, *Tr. Lewisi* in culture presents essentially the same two types. It has multiplication rosettes and free-swimming forms corresponding to the spirochetes, though much wider. This is the case also with several of the other species, as will be shown presently. In our opinion there can be no doubt but that the two forms belong to one species.

It will be remembered that the blood of the birds from which this type was obtained had, in several instances, two forms of trypanosomes—the small spindle—and the long S-shaped trypanosomes. The question naturally arises as to what relation exists between the two forms in the blood and the two forms in the cultures. It is possible that the large S-form is the source of the rosette and that these represent the female cell, while on the other hand the spindle-shaped trypanosome of the blood gives rise to the spirochete which may be considered as an indifferent, or asexual, form. While the latter are capable of multiplying rapidly, they probably do not change into the other type. On the other hand it would seem as if some of the rosette cells could differentiate into the free form. Such a transformation, however, has not been observed.

Sub-type 1a. This can be easily confounded with the preceding. A close examination however, shows that there are some marked differences. The rosettes appear to predominate, and by their number and size attract attention much as they do in the case of *Tr. Lewisi.* The free-swimming forms are much shorter than those of the preceding type, and are distinctly wider near the anterior end. This gives them a slight tapering appearance. They are very actively motile, have clear contents, and agglutinate by their posterior ends. They may be regarded as intermediate between Type 1 and the following, especially Type 4. This type was met with in strains A, B, U, and Z.

Type 2. This was met with but once and then in the blood of a hawk. We have named this species, as already stated, *Tr. Mesnili.* Not only is the trypanosome as found in the blood different from *Tr. avium*, but an even greater difference can be seen in the cultures.

Cultures made from the heart-blood showed on the third day several groups of spherical or oval bodies which measured five to eight μ in diameter. They contained a hyaline plasma, somewhat vacuolated and very highly granular, with numerous bright refractile globules of a greenish tint. These globules were about 0.5 μ wide. The cells composing this group showed a long free flagellum on the outside of the mass. A wide, slowly waving, undulating membrane could also be seen. In addition to this group a number of free, motile, coarsely granular trypanosomes, about 16 μ long, were observed.

On the sixth day the culture was fairly rich, and presented a very striking appearance. The free, swarming forms were numerous, and traveled about very rapidly, whip foremost, at times rolling on their long axis. The body of the cell was from 18–21 μ long and about three μ wide. The greatest width was near the anterior end which narrowed rather abruptly and terminated in a long free whip, From the point of greatest width the body tapered gradually toward the posterior end which was either pointed or slightly blunt. Numerous bright globules or refractive bodies filled the posterior two-thirds of the body. Large, rounded, rolling forms are also common.

The actively motile, free forms showed a tendency to agglutinate in a manner highly suggestive of *Tr. Brucei.* That is to say, the cells adhered not so much by their posterior extremities, but by their sides, thus forming irregular groups of 6–10 or 20 cells. As with *Tr. Brucei*, these agglutinated cells have their whips on the outside (Fig. 4, Plate 5). Frequently they attach themselves to red blood cells.

In addition to the agglutination groups, real multiplication rosettes are to be seen, though rather few in number. These, as in the case of *Tr. Lewisi* and *Tr. avium*, have their flagella on the inside (See Figs. 2 and 3, Plate 5). The older rosettes present

a very characteristic appearance. The spindle-shaped or pyriform cells are on the periphery, while the central portion, 10 to 15 μ in diameter, shows a tangle of slowly waving flagella.

Further details regarding this species will be given under *Tr. Mesnili.*

Type 3.—This was found in a goldfinch, No. 353, in the stained blood of which only one trypanosome could be detected. As seen from Table II the measurements of this cell correspond closely with those of the small form of *Tr. avium*, so much so that if this were the only criterion there would be no hesitation in regarding it as of that species. However, the coarse granulation of the contents, as seen in Fig. 1, Plate 6, suggests a difference, and this is clearly substantiated by the results of cultivation. In view of the fact that this type is so clearly distinct from either *Tr. avium* or *Tr. Mesnili*, we must look upon it as a new species, and have named it, as already mentioned, *Tr. Laverani*, in honor of Dr. Laveran of the Pasteur Institute.

This trypanosome is characterized by a very slow growth in cultures. In such the flagellates are scarcely to be seen before the fifth day; they are moderately abundant about the tenth or twelfth day, and cannot be said to be rich until about the second or third week. Thus, the blood of the bird was planted on two tubes of the medium. Ten days later, each tube showed several rosettes and some actively motile trypanosomes. The maximum of growth was reached shortly before the twenty-third day, for at that time the growth was quite rich, but the motion had disappeared or nearly so. An examination on the thirty-fifth day showed masses of round bodies, many of which were also single. Tangles of free flagella were also common.

The slow growth in cultures, of *Tr. Laverani* distinguishes it at once from either of the preceding types. There are, however, other equally striking differences.

The rosettes are less common than in Type 2 and are perhaps smaller. They are composed of rounded, pyriform, or spindle-shaped cells which contain numerous bright yellowish-green globules, which are larger, and perhaps more abundant, than those of the preceding species. The cells taken as a whole are narrower

than those of *Tr. Mesnili.* Their flagella, directed centrally, are more delicate and hence more difficult to see in the living preparation than are those of the preceding type (Fig. 3, Plate 7).

The free forms, while common enough, are much less motile than is the case with either of the preceding. It may often happen that in a preparation not more than a dozen cells can be found to travel about actively. When moving about they roll on their long axis, whip forward. Usually they attach themselves by their whips to the glass and show a slow swaying motion.

The absence of marked motion is apparently due to a stickiness of the exterior of the free cell. This is seen in the fact that a red blood corpuscle often adheres to the middle portion of the cell when touched by the latter. It is further indicated by the marked tendency to form agglutination groups, which are somewhat suggestive of those of *Tr. Brucei.* The two cells may adhere laterally, with the whips at opposite ends, and the group thus started may increase in numbers so that eventually several hundred cells may be together. In such groups the whips are on the outside and in active motion. The cells usually contain from 5–10 large globules which are found in the posterior end though not always.

Rounded, rolling forms about the size of a red blood corpuscle (Fig. 6, Plate 7) are common in the early cultures and represent dividing forms. In old cultures, the rounding up, as with other trypanosomes, is a common involution change.

The stained preparations show a coarsely granular nucleus, and close to it or anterior is a small blepharoplast. A most interesting feature is the presence of a terminal rod which we have not observed thus far with certainty in any other type. This rod usually lies right up against the wall at the posterior tip of the cell, and for that reason can be easily overlooked. In such a case, all that can be observed is a slightly deeper stain of the edge of the cell at that point. When, however, cell-division is taking place and especially if the preparation is thoroughly flattened out in the act of spreading, this body can be readily seen (Plate 6). That this terminal rod is a distinct structure cannot be doubted. It divides about the same time or a little later than does the

nucleus, but does not approach the latter except possibly in the rounded forms. It is stained a lighter pink by the Romanowsky method than is the nucleus. Thus far we have been unable to make out any connection with the other structures of the cell. It is possible that a similar body is present at the anterior end, as suggested by some of the photographs.

In a recent paper on the development of Herpetomonas, Prowazek[75] describes a flat, spiral thread which passes from the blepharoplast to the posterior end of the body where it terminates in an "undeutlichen Doppelkorn." In the process of reduction previous to copulation this diplosome was said to divide, giving rise to a group of four granules, two of which divided again, so that a group of six granules resulted. The further changes of these bodies could not be followed.

As stated above we have looked closely for some connection with the other structures of the cell, but have not been able to observe any. Possibly, by giving special attention to the method of staining, something may be ascertained on this point. In the dividing cell two such rods are found, but whether the division occurs transversely or longitudinally cannot be said. This terminal rod can be made out more or less easily in the majority of the cells. There are some, however, in which for some reason this cannot be done.

The staining of the cultures of this type is much more difficult than of either of the preceding. The globules which are seen in the living cell do not readily stain, and hence may appear as colorless bodies or vacuoles. On allowing the stain to act for a longer time the globules may be stained a dark red, but in that case the nucleus, blepharoplast, and terminal rod cannot be made out in the deeply stained contents.

Sub-type 3a.—This was obtained from a brown thrasher, No. 386, and is designated as strain T. It is certainly closely related to, if not identical with, *Tr. Laverani.* Like the latter it is a slow grower, requiring from 12–14 days to yield a fair culture. Rosettes are common and like the preceding are made up of round bodies or spindles containing many large globules. Free round bodies 8–10 μ in diameter are to be seen rolling about in

early cultures. The free forms 15–18 μ long, unlike those of the preceding, have a narrow posterior end, are narrower and more tapering, and possibly more coarsely granular; they have a smooth gliding motion somewhat circular in course. They apparently are also quite sticky, since they tend to stick to the glass or to agglutinate in groups of 10–20 or more cells. The agglutination is perhaps not as marked as in the case of *Tr. Laverani.*

The length of the body of the free form is on an average 15–18 μ; at times cells 25 μ long are met with. The width varies from 2.5–4 μ. The free whip is from 5–10 μ long, but in the case of the round form it may be much longer. The blepharoplast is very small, usually not over 0.3 μ, and is anterior to the nucleus. The posterior end tapers to a point, whereas the anterior end is commonly blunt. The contents, especially in the anterior half, are rich in granules which stain deeply.

The round forms are, as a rule, about 6 μ in diameter. The smaller ones on division may give rise to cells that measure but $3 \times 5\,\mu$. These usually have a relatively long whip, 10–15 μ long. There is a faint suggestion of a terminal body, as in the case of *Tr. Laverani*, but further staining will be necessary to positively demonstrate this structure. The nucleus is apparently more difficult to stain than is that of the other types.

Type 4.—This was met with three times, and in cultures only. It was obtained twice from blue jays (Nos. 273 and 429), strains M and C′; and once from a rusty blackbird (No. 420) strain A′. In these cases the blood preparations, even on re-examination, failed to show the native trypanosome.

The cultural characteristics of the three strains were practically identical, and for that reason they may be taken to represent a single species. On the other hand, they present marked differences from the preceding types, sufficiently so, to entitle them to be considered as representing a distinct species.

The growth is very rapid, and as a result it is quite rich on the fourth or fifth, and reaches its maximum on about the eighth day. It shows the usual two forms—rosettes and wide, free-swimming cells.

The rosettes are by no means as marked as with the other types. They are scarce and small, rarely consisting of more than about 20 cells. They may be composed of round bodies or of elongated spindles. The whips are on the inside. The spindle forms sway quite freely, sufficiently so as to detach themselves, and it is probably due to this that the rosettes are so few and small. The round bodies and spindles show only a fine granulation of the contents; and are thus in marked contrast to the two preceding types. The round bodies are not much larger than a red blood cell.

The free forms are numerous and move about very rapidly, whip forward. When in motion the cell turns on its long axis, and as a result it may have a wave-like appearance or lateral sway. The body is usually about 15 μ long, and is widest near the anterior end. The width is 3 μ or less. The posterior half may contain numerous small granules, unlike the large globules of *Tr. Mesnili* and *Tr. Laverani*. The undulating membrane can be readily seen at the anterior end.

Agglutination occurs as with the former two types by several cells sticking together lengthwise, the whips remaining on the outside. Large masses of cells may thus form. That this is largely due to a stickiness of the wall is shown by the readiness with which they attach themselves to red blood corpuscles. At times a single cell may be seen to travel about with two such corpuscles.

Sub-type 4a.—This is represented by strain X from goldfinch No. 354. It resembles the preceding but shows considerable variation. The rosettes are even scarcer and are rarely composed of more than a dozen cells which sway actively.

The free form is about 20 μ long and 3 μ wide. Smaller forms of 14–16 μ and larger ones of 24–26 μ are also present. The free whip is from 14–22 μ long. The relatively large blepharoplast (1–1.3 μ) is almost always on the side of the nucleus which is about 2 μ wide. This position of the blepharoplast and its large size distinguishes the cell at once for *Tr. Laverani*. The posterior part of the body is blunt, while the anterior part elongates along the whip to a fine point.

DESCRIPTION OF SPECIES.

Before taking up the description of the species met with by us it will be well to consider briefly the several forms found by other observers and endeavor to correlate their findings as far as possible with our own. This is obviously a matter of some difficulty, owing to the entire absence of cultural characteristics.

The earliest observations, those of Danilewsky, indicated the presence of two forms of trypanosomes in the blood of birds, the small and the large form. As shown heretofore, Danilewsky was undoubtedly correct in the general assumption that these represented but one species, his *Trypanosoma avium*. The close agreement in form and size of his organisms with the majority of the trypanosomes studied by us leads us to accept them as identical, and for that reason we have designated our common species by the old name.

The description of *Tr. avium* as given by Laveran and applied to the organism found in an owl can, in the main, be reconciled with the above. The length, including the whip, is given as 33–45 μ. This, it will be seen, corresponds to the smaller forms mentioned, which measure, without the whip, 20–25 μ. The position of the centrosome, near the posterior end, accords better for the small form than for the large one.

The form which Hanna met with in the blood of pigeons, in India, certainly resembles very closely the large type, such as is shown in Plate 3, and may therefore be looked upon as likewise falling under *Tr. avium*. The same may be said of the trypanosome which he describes as present in the Indian crow, but which Dutton and Todd apparently refer to as in a blue jay.

The short, broad trypanosome which Dutton and Todd found in Senegambia is in form, size, and position of the centrosome the same as the small forms found by us, as will be seen on comparing our photographs with their illustration. It can therefore be regarded as *Tr. avium*.

The *Tr. Johnstoni* of Dutton and Todd certainly represents a distinct species. We have found one single specimen, in a stained preparation of the blood of the woodpecker, No. 391, which resembled somewhat this species. Unfortunately the slide

was misplaced, and as no cultures were made we are unable to give any detail.

The most recent species of bird trypanosome is the *Tr. paddæ*, described by Laveran and Mesnil, and by Thiroux. Were it not for the fact that this organism is said to have a very short free whip, it might well be taken to correspond with the large S-form which is finely striated, as shown in Plate 3. It is to be hoped that cultures of this organism will be made, in which case it will be possible to make an exact comparison with those of ours.*

TRYPANOSOMA AVIUM, DAN., N. & MACN. EMEND.

This is apparently the most common species found in birds, having been met with twenty-five times in the blackbird, blue-bird, blue jay, oriole, robin, English sparrow, and song sparrow. This does not include the sub-type 1*a*, previously mentioned, which was found four times, namely, in the mourning dove, blue jay, and flicker.

The native trypanosome may appear in either the small or the large form, or in both. These two forms have already been described, and for that reason, in order to avoid repetition, the reader is referred to that portion of the paper.

It grows very readily on the blood agar medium, and, as a rule, rich cultures are obtained on the fourth to the sixth day. The culture is characterized by the presence of two strikingly different forms, the multiplication rosettes and the free-swimming, darting, thread-like spirochetes.

The rosettes are made up of smaller cells and are a more prominent feature of the culture of *Tr. avium* than are the like formations in the case of the other species. The long, slender spirochete form is apparently distinctive, since the corresponding free-swimming forms of the other species are shorter and wider. Moreover, the blepharoplast in the latter is by the side of or

*As this paper is going to press we learn from Dr. Thiroux that he has succeeded in cultivating this trypanosome which is now in its third generation. An examination of some preparations made from these cultures and kindly sent to us by him show at a glance that this organism is entirely distinct from our Tr. avium. The cultural forms more nearly approach those of Tr. Laverani. Dr. Thiroux has also shown that the injection of Tr. paddae into canaries and paddas is not followed by a halteridium infection and hence that these two organisms are entirely distinct, thus confirming our results and the view expressed at head of page 273.

anterior to the nucleus, whereas in the spirochete it is considerably posterior to this structure.

An extended description of these two forms is given on pp. 282 and 283.

The attempts at infection of birds with cultures of this trypanosome were far from satisfactory. It should be stated, however, that most of these inoculations were made with the object of finding intracellular parasites, which according to Schaudinn's views should be expected, and consequently the search for the trypanosomes was not as thorough as it might have been. The culture fluid used for the injections was either derived from a single strain, or in some instances a mixture of several was used. The injections were as a rule intrapleural, and the fluid was always extremely rich in trypanosomes. The usual dose was from 0.1–0.2 c.c. One screech owl, two pigeons (squabs), three chickens (ten days old), seven robins, twenty-two sparrows, and six old canaries were used. The work with robins and sparrows was rather unsatisfactory on account of the difficulty of keeping the birds alive for any length of time. In only one of these birds, a canary, was a positive result obtained.

This canary received an injection of a very rich culture of strain D. Four days later, it showed a marked leucocytosis, and three trypanosomes were found in the fresh blood. These were in the form of a long, straight spindle, about 4.5 μ wide and 28 μ long, with a free whip measuring 12–15 μ. The undulating membrane was very conspicuous, starting from near the posterior end and passing over the body in two large waves. The nucleus was readily visible as a large oval, about as wide as the parasite. Fine granules were present, especially in the posterior part. The motion was rather sluggish and confined to the neighborhood of the one field of the microscope. The next two examinations, made about a week apart, were negative. On the third and fourth week a single trypanosome was found each time. It then appeared to be smaller than on the previous occasion. The body was about 17 μ long, three to four μ wide, and the whip measured about eight μ. Three examinations in the course of the next two weeks were again negative. The trypanosomes were thus

detected, off and on, for eleven weeks, and were found in the blood at the time of death. Although many attempts were made to obtain stained preparations of the trypanosome, they all failed, owing to the extreme scarcity of the parasite. Immediately after death cultures were made from the heart-blood of this canary, and in four days they showed a rich growth, consisting of the typical rosettes and spirochetes of *Tr. avium.* This culture, known as strain Y, is not included in Table I, since it was derived from an artificially infected bird. It makes the thirtieth strain isolated from birds.

TRYPANOSOMA MESNILI, N. SP.

This trypanosome was found in the blood of a red-shouldered hawk (*Buteo lineatus*), associated with *halteridium* and *H. Ziemanni.* The organism was very scarce, since only one specimen could be found on four slides, each of which was systematically searched by means of a movable stage.

The native trypanosome (see Fig. 1, Plate 5) is characterized by its large size and great bulk, and by a wide, rounded posterior extremity. The length of the body is 50 μ, and the width opposite the nucleus is 8 μ. The width of the cell at the posterior end is 5 μ. The distance from the posterior end to the centrosome is 7 μ, and from this to the nucleus it is 10 μ. The anterior portion of the body, which is bordered by a thick, undulating membrane, tapers rather abruptly to a narrow end which undoubtedly terminates in a free flagellum. This, however, cannot be made out in the specimen. The trypanosome was not seen alive in the blood.

The contents of the cell stain heavily and are coarsely granular. The centrosome is round and about 1 μ wide. The nucleus is relatively small and is found near the side having the undulating membrane.

The cultures of this organism grow very rapidly, and are very rich on the sixth to the seventh day. After that they undergo involution changes, forming large masses of very granular, round bodies. A few motile cells may be met with in such cultures, even at the end of six weeks.

The cultures show two types of cells, resembling in a general

way those met with in *Tr. Lewisi* and *Tr. avium.* The one type makes up the multiplication rosette, while the other is free and swarming, and corresponds to the spirochete stage of *Tr. avium.*

The multiplication rosettes (see Figs. 2 and 3, Plate 5) vary considerably in size. The smaller ones are about 10 μ in diameter, and are made up of cells in process of active division. Such cells are about 3 μ wide and 5–6 μ long, and on division yield cells which are about 2 μ wide and 4 μ long. On account of the rapid division, the free flagella are very short and can scarcely be made out.

The larger rosettes consist of more fully developed cells, 10–100 in number. These are usually about 10 μ in length, but they may attain a width of 6 or even 7.5 μ. The full-sized spindles measure 4×15 μ. The free whips, about as long as the cell, are easily seen in the central portion of the rosette. On account of the length of the whips the cells composing such rosettes exhibit a marked swaying motion. The large oval or pyriform cells stain deeply, and are filled with dark granules and bright refracting globules. When free, as is often the case, they may be seen rolling about in the fluid.

The free, swarming cells, as stated above, correspond to the spirochete stage of *Tr. avium.* Unlike the spirochetes of the latter, they are short and very wide, and are provided with very long, free flagella. Such free cells ordinarily measure 4–6 μ in width and 20–25 μ in length, not counting the whip, which often measures 17–20 μ. From the widest portion, near the anterior end, the body gradually tapers toward the posterior extremity, which is either blunt or slightly pointed. On account of their large size, the undulating membrane can be seen at the anterior end, and for the same reason these cells have a stately, gliding motion. They travel rapidly, whip foremost, either in a straight line or in a circle, and often may be seen to roll on their long axis.

When the growth is very rapid, as a result of a specially favorable medium, the cells show only very minute globules, scattered all through the plasma. At other times the granules are

rather large, and have a distinct yellowish green color. They are most abundant in the posterior half of the cell.

We have pointed out in connection with our other work on the cultivation of trypanosomes that the presence of bacteria tends to destroy these protozoa. The result is largely dependent on the kind of bacteria which chance to be present. In one instance, Laveran and Mesnil found the culture of *Tr. Lewisi* to remain alive and virulent in the presence of bacteria for about two weeks. A similar result, extending over a much longer period of time, we have noted in connection with *Tr. Mesnili.* A culture of this organism developed a contamination with a slow-growing streptococcus, and inasmuch as the trypanosome continued to grow, even luxuriantly, it was transplanted and subcultures were kept up. These have now been maintained for over three months, and, if anything, the mixed cultures are somewhat better than the pure ones.

The free, swarming trypanosomes show a considerable tendency to agglutination. This usually begins by two cells overlapping at their posterior ends. Other cells attach themselves to these by their sides, and thus large masses soon form. At times this agglutination takes place so rapidly under the cover-glass that, in the course of an hour or two, nearly all of the cells gather into these irregular clumps. In such agglutination groups the flagella are always on the outside. The undulating membranes and whips continue their motion, and as a result the entire mass can be seen to travel through the field.

The fact that the cells agglutinate along their whole length would seem to indicate that the surface of the body is more or less sticky. This view is substantiated by the behavior of these cells with respect to blood corpuscles. In some cultures it is a common occurrence to find the trypanosomes moving rapidly about with a red blood cell attached to the median portion. This could hardly happen unless the outside of the trypanosome was somewhat gelatinous. There is also a tendency, especially for the larger forms, to attach themselves by means of their whip to the glass surface.

The free motile forms can be seen in all stages of division.

For this purpose the cell first shortens to a large pyriform body about 10 μ wide and 15 μ long. The whip likewise shortens. The blepharoplast divides first, then the nucleus, and finally the body itself begins to split (Fig. 5, Plate 5).

Only two injections of cultures of this trypanosome were made, and both were negative. A young screech owl (*Megascops asio*) and three young chickens (10 days old) were used.

TRYPANOSOMA LAVERANI, N. SP.

This interesting species was met with but once and that in goldfinch, No. 353. It was overlooked in the examination of the fresh blood and in the stained preparations. When, however, the culture was obtained from the heart-blood the original stains of the blood were re-examined by the help of a movable stage, with the result that one trypanosome was found on three slide smears.

The native trypanosome is shown in Fig. 1, Plate 6. It is a wide spindle which measures 20 μ in length and 6 μ in width. The blepharoplast is close to the sharp-pointed posterior end, about 1 μ distant. The photograph shows no free whip, but this must be considered as due to defective staining since in the cultures a long free flagellum is easily observed. The posterior part of the cell is coarsely granular which peculiarity is brought out very clearly in the cultures. The wide nucleus occupies the middle of the cell.

In artificial culture the trypanosome is characterized by a very slow and sparse growth.

The multiplication rosettes are rather few in number and are small. They consist of rounded or pyriform bodies, which eventually elongate to oval or spindle-shaped cells. In the living preparation these are found to contain numerous granules and bright yellowish-green highly refracting globules which do not readily take the dye, and consequently in stained preparations appear as colorless globules (see Plate 7). The globules are usually about 1 μ in diameter, but at times they may be found twice that size.

The spherical forms which may be looked upon as giving rise to the rosette are frequently found free and in active division.

The blepharoplast divides first, then the nucleus, and lastly the protoplasm. The division is longitudinal and uneven, that is to say, the young cell may be only half as wide as the remaining part of the parent cell. The latter may therefore pass on to a second or third division, and, as a result, groups of three or four of such cells are often found. Such round or oval bodies usually measure $8 \times 10 \mu$, but may attain a size of $12 \times 14 \mu$. A noticeable feature in these bodies is the terminal rod which usually lies against the posterior wall, but may at times approach the nucleus. The free whips on these forms are relatively short, about $10–14 \mu$.

The free forms appear, either as slender spindles, which have a fairly constant width along the entire length of the body, or as spindles which are appreciably wider in the middle portion and taper toward both ends. The latter are from $14–20 \mu$ in length and $4–5 \mu$ in width, and have a free whip which is as long or even longer than the body. The posterior end may be sharp but more often is blunt or cut off square, and shows the peculiar terminal rod. The slender spindle, mentioned above, measures from $20–25 \mu$ in length and $2.5–3 \mu$ in width. The whip is relatively shorter than in the preceding type, measuring about $10–14 \mu$. In both forms the blepharoplast which is comparatively small lies anterior to the nucleus. It is usually about 0.7μ long, but in the dividing forms it may attain a length of nearly 2μ.

The free form divides by longitudinal fission without shortening or rounding up to any appreciable extent. The blepharoplast divides first, and this leads to the formation of a new whip and then, very often, to a division of the anterior portion of the cell. The nucleus then divides and the cleavage continues until complete division results. The terminal rod apparently divides after the nucleus, and, at the stage of complete division, two of these bodies can be seen at the posterior end.

The relatively feeble motion and the marked tendency to agglutinate have been mentioned under Type 3.

No inoculation experiments have as yet been made with this culture.

UNNAMED SPECIES.

The three strains described under Type 4 without doubt represent a distinct species, but inasmuch as the native trypanosomes

have not been found, even after repeated search of the stained preparations of the blood, it has not seemed desirable at present to give this type a definite name. Inoculation experiments are now in progress and may help to supply this deficiency.

As to the sub-types 1*a*, 3*a*, and 4*a*, the opinion may also be expressed that they belong to new species. It has not, however, been possible up to the present time to give these a very thorough study, especially of the stained preparations of the cultures, and consequently they must be reserved for another occasion.

RELATION OF TRYPANOSOMES TO THE CYTOZOA.

This subject has acquired especial importance in view of the work of Schaudinn on the halteridium and the "leucocytozoön" of Danilewsky. The main conclusions arrived at by this investigator have been given in the introduction, and as already indicated they are not substantiated by our work.

In the first place we have shown that trypanosomatic infection of birds is widespread. Moreover, there is sufficient evidence of the existence of a large number of species of trypanosomes, and, as can be seen in Table I, a given species of bird (bluejay, goldfinch) may be subject to infection by two or three kinds of trypanosomes.

Double, triple, or even quadruple infection in birds is not an uncommon occurrence, and on that account it is not surprising to find trypanosomes associated with one or more intracellular parasites. Such association, however, cannot be said to have any other significance than that of mere chance or accident. The two extremes: first, of cytozoa without trypanosomes; and second, trypanosomes without cytozoa, are common enough as has been shown. Furthermore, it has been made evident that a given cytozoön, as, for example halteridium, may be associated with at least three distinct species, *Tr. avium*, *Tr. Mesnili*, and *Tr. Laverani*. Again, an examination of Table I will show in the case of the robin that the same species, *Tr. avium*, may be associated with any one of four cytozoa. The remarkable uniformity of the occurrence of this species of trypanosome under these conditions is difficult of explanation from the stand-

point taken by Schaudinn. It is also worth noting that in the one case of infection with the "leucocytozoön" *H. Ziemanni* we have obtained a trypanosome, *Tr. Mesnili*, which in cultural characteristics is totally different from the *Spirochete Ziemanni* described by Schaudinn as a stage of this cytozoön.

In view of the ease with which trypanosomes can be cultivated in the test-tube it is reasonable to suppose that the same result can be obtained with the mosquito. That is to say, the few trypanosomes which may chance to be present in the blood sucked up by the mosquito rapidly multiply in the stomach of the insect and give rise to rich cultures similar in every respect to those met with in the test-tube. Thus, the multiplication rosette, with the whips directed centrally, will be found as the predominating feature with a given species of trypanosome. In the case of *Tr. avium* the long, slender, free forms or spirochetes are especially noticeable.

It seems to us, therefore, that the observations of Schaudinn are open to an entirely different interpretation than that given by him. It appears that he has cultivated trypanosomes *in vivo* and has obtained forms which agree fully with those obtained by us in artificial culture, *in vitro*.

Assuming the identity of the flagellates found by Schaudinn and ourselves *in pure culture*, it can be expected that like results would be obtained by the injections, on the one hand, of suspension of the mosquito culture, and on the other hand of the culture fluid from the test-tube. On this point we have made a very large number of inoculations into sparrows, robins, owls, canaries, young pigeons, ring doves and very young chickens, but have never been able to obtain any indication of the development of intracellular parasites as a result. The amount of culture fluid thus injected into a bird was incomparably greater than could be obtained by the injection of a large number of mosquitoes. It is true that with one exception we failed at the same time to infect the birds with trypanosomes.

Since the injection of suspensions of the infected mosquitoes, or the mere bite of a few of these insects, as shown by Schaudinn and by the Sergents, is capable of causing an infection with the

intracellular parasites, such as the halteridium, it follows that one of two conclusions can be drawn. First, it may be held that the flagellates observed by Schaudinn in the mosquito are the real stages of the cytozoön and that they are wholly distinct from the trypanosomes found in birds. Or, second, it may be held that the flagellates found in the mosquito are derived from the trypanosomes in the birds, and as such have nothing to do with the intracellular parasites; in which case the positive infection with the mosquito must be due to some as yet unrecognized stage of the cytozoön. This latter view is, in our opinion, the correct one.

In order to furnish a definite solution of this question it will be necessary to allow mosquitoes to bite birds which by the cultural method have been shown to be free from trypanosomes. If flagellates appear in the stomach of such mosquitoes, after feeding on birds known to be free from trypanosomes, it will be justifiable perhaps to consider them as stages in the development of cytozoa. On the other hand, since only about 10 per cent of the mosquitoes which feed upon the infected bird develop flagellates (according to the Sergents about 25 per cent), it ought to be possible to produce with those which show no trypanosomes the cytozoön infection.

The recent detection by Rogers[76] of trypanosomes in so-called culture, made with the blood of patients infected with *Piroplasma Donovani* is assumed by some to indicate that the trypanosome is a stage in the development of that parasite. This position, however, is far from being established. It may well be asked, in view of the great frequency of trypanosomatic infection of birds, whether or not a similar condition may not obtain with man, especially in the tropics. The direct detection of such trypanosomes in the blood would be perhaps as difficult of accomplishment as it is in the case of birds, and for that reason they could be easily overlooked. It is probable that if systematic cultivations are made of the blood of a large number of individuals inhabiting the warm countries, conditions will be found to parallel the infection of birds, rats, fish, and amphibians. On *a priori* grounds one could expect to find relatively harmless trypanosomes in the blood of man besides the one pathogenic *Tr. gam-*

biense. The recovery from trypanosomatic infection of the one known case, that of a European, may be looked upon as due to the presence of a different trypanosome from that of sleeping sickness. Similarly, the observation of Rogers may be interpreted as indicating the existence of a hitherto unrecognized form of trypanosomatic infection in man.

SUMMARY.

The main results arrived at in this investigation can be briefly summarized as follows:

1. Trypanosomatic infection of birds is very common and widespread.

2. The different species of birds may harbor different species of trypanosomes; and the same species of bird may be infected by several species of these parasites.

3. The trypanosomes may or may not be associated with intracellular parasites; and no constancy can be shown to exist between a given trypanosome and a given cytozoön.

4. The *Trypanosoma noctuæ* and the *Spirochete Ziemanni* of Schaudinn probably represent trypanosomes which have multiplied in the mosquito; and are not to be considered as stages in the life-history of cytozoa.

5. The unsuspected frequency of trypanosomes in birds makes it probable that, in the tropics, animals and even man may be found to harbor in small numbers such parasites. The presence of trypanosomes in the blood of man does not necessarily mean an infection with *Tr. gambiense.*

In conclusion it is a pleasure to acknowledge the aid given by the Rockefeller Institute in the furtherance of this work. Our thanks are also due to Dr. J. F. Eastwood for his great help in the photographic room, and to Mr. H. N. Torrey, the present holder of the Rockefeller fellowship, for his invaluable assistance in the work of cultivation and staining.

EXPLANATION OF PLATES.

These illustrations were taken by means of the large Zeiss microphotographic apparatus. With the exceptions noted the objects are all magnified 1,500 diameters. They are all stained by the Romanowsky method, except Fig. 5, Plate XI, in which case a living preparation was used.

PLATE 1.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium.*

FIG. 1.—Trypanosome giving rise to strain A, in mourning dove, No. 5.

FIG. 2.—The same of strain B, in mourning dove, No. 6.

FIG. 3.—The same of strain C, Baltimore oriole, No. 41.

FIG. 4.—Another slide of the same blood as preceding, showing the crushed form.

FIG. 5.—Trypanosome of which no culture was obtained, from Baltimore oriole, No. 272.

FIG. 6.—Another slide of the same blood as the preceding, showing a slightly crushed form. Note flagellum.

PLATE 2.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium.*

FIG. 1.—Trypanosome giving rise to strain G, robin, No. 50.

FIG. 2.—The same of strain D, robin, No. 51.

FIG. 3.—The same of strain F, robin, No. 54.

FIG. 4.—From same preparation as preceding, showing the trypanosome slightly flattened. To the left a cell infected with a female form of *Hæmoproteus majoris*, Lav. This shows in the center a faintly stained nucleus, while adjoining and overlapping it is the large deeply stained blepharoplast.

FIG. 5.—Small form of trypanosome, of which no culture was obtained, from robin, No. 270.

FIG. 6.—Large S-shaped trypanosome in the same preparation as the preceding. The undulating membrane is particularly well developed. The nuclear portion is not stained.

PLATE 3.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium.*

FIG. 1.—Small form of trypanosome giving rise to strain K, in blue jay, No. 244.

FIG. 2.—Another preparation of the same blood showing the crushed form.

FIG. 3.—Large trypanosome S-shaped in the same preparation as that of Fig. 1. The blepharoplast, surrounded by achromic zone, is near the nucleus. Note the striations on the surface of the cell.

FIG. 4.—Large form in same preparation as preceding.

FIG. 5.—Small form of trypanosome of strain R, in blue jay, No. 278. Note the free flagellum.

FIG. 6.—Large S-shaped trypanosome in same preparation as preceding.

PLATE 4.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium.*

FIG. 1.—Trypanosome giving rise to strain E, in robin, No. 53.

FIG. 2.—The same of strain H, in song sparrow, No. 142.

FIG. 3.—Small form of trypanosome, of which no culture was obtained, in song sparrow, No. 288.

FIG. 4.—Large form in same preparation as preceding.

FIG. 5.—Trypanosome, of which no culture was obtained, in blackbird, No. 401.

FIG. 6.—Another in same preparation as preceding.

PLATE 5.—NATIVE AND CULTURAL FORMS OF *Tr. Mesnili*, n. sp.

FIG. 1.—*Tr. Mesnili* in blood of hawk, No. 350, source of strain P.

FIG. 2.—Culture of *Tr. Mesnili* on blood-agar, gen. 6, five days at 25°. Small multiplication rosette consisting of large and small cells, some in process of division. The flagella are central and short.

FIG. 3.—From same preparation as preceding. Large multiplication rosette with full-grown cells, some dividing. The flagella are long and centrally directed.

FIG. 4.—From same preparation as preceding; agglutination group of five swarming cells. Reduced from 1500 × to about 1160 ×.

FIG. 5.—From same preparation as preceding. A free-swarming form, like that shown in Fig. 4, in process of division. In the center are two faintly stained nuclei while the two blepharoplasts are deeply stained.

PLATE 6.—NATIVE AND CULTURAL FORMS OF *Tr. Laverani*, n. sp.

Note in the latter the presence of the terminal rod at the posterior end; also the smallness of the blepharoplast and its position, anterior to the nucleus.

FIG. 1.— *Tr. Laverani* in blood of goldfinch, No. 353.

FIG. 2.—Free form with blunt end, culture 12 days at 25°, Gen. 6.

FIG. 3.—Free tapering form. From same slide as preceding.

FIG. 4.—The slender or narrow free form. This and the next two are from a culture in Gen. 7, grown six days at 25°.

FIG. 5.—The free form in process of division; note the two nuclei, two blepharoplasts, and two flagella; also the presence of colorless globules.

FIG. 6.—Dividing form, with two nuclei and two blepharoplasts; each of the latter is in process of division as shown by the fact that each gives off two flagella. Note the prominent terminal rods.

PLATE 7.—CULTURAL FORMS OF *Tr. Laverani.*

These were obtained from the seventh generation grown at 25° for 6 days. Note the prominent terminal rod and the position of the blepharoplast, anterior to the nucleus; also the presence of the colorless globules.

FIG. 1.—Dividing free form with one nucleus and two blepharoplasts.

FIG. 2.—The same as Fig. 1, but with two nuclei and two blepharoplasts. Compare this and the preceding figures with Fig. 5, Plate 5, and Fig. 1, Plate 11.

FIG. 3.—Multiplication rosette of pyriform and spindle-shaped cells. Note the tangle of flagella at the center.

FIG. 4.—Large and small oval bodies, the former in process of division. Note that each blepharoplast is giving off two whips, which indicates that another division is about to occur.

FIG. 5.—Like Fig. 4, but the division has gone on resulting in the formation of four nuclei, and four blepharoplasts, and four whips.

FIG. 6.—Earlier stage of the round form than is shown in Figs. 4 and 5. One nucleus, two blepharoplasts, and two whips.

PLATE 8.—CULTURAL FORMS OF *Tr. avium.*

Strain Q from robin, No. 257, gen. 7, grown seven days at 25°. The figures on plates 8, 9, and 10, and figs. 1, 2, 3, 4 of Plate 11 were made from the same slide.

FIG. 1.—Early type of multiplication rosette consisting of round bodies. Shows whips on the inside.

FIG. 2.—Another multiplication rosette, cells oval in form. One spirochete in the field. Whips as before.

FIG. 3.—Another multiplication rosette of short, narrow spindles. Whips as before.

FIG. 4.—Large multiplication rosette of fully developed spindles. Whips as before. Some cells dividing.

FIG. 5.—Small rosette from same preparation as preceding. The two rounded cells are in process of division as seen by the two stalks or whips which each gives off; also by the partial division of the blepharoplast.

PLATE 9.—CULTURAL FORMS OF *Tr. avium.*

From the same preparation as before. The figures show the initial stage of rosette formation.

FIG. 1.—The remnant of whip shows on each cell. The smaller cell has not begun to divide, the larger shows two nuclei and two blepharoplasts; one of the latter is dividing.

FIG. 2.—Large form as before, with four nuclei and five blepharoplasts.

FIG. 3.—Rosette of several such bodies.

FIG. 4.—Two cells resulting from the division of the round form.

FIG. 5.—Early stage of rosette consisting of four cells. Beside it a spirochete.

FIG. 6.—Rounded form, possibly of a spirochete, shows three nuclei and three blepharoplasts deeply stained. The lines on the surface are probably short flagella.

PLATE 10.—CULTURAL FORMS OF *Tr. avium.*

From same preparation as before. The figures show the swarming spirochete type.

FIG. 1.—A group of spirochetes.

FIG. 2.—A very long spirochete (50 μ) and a short one.

FIG. 3.—Agglutination of two spirochetes, showing the overlapping of the posterior ends.

FIG. 4.—Another agglutinated pair of spirochetes; the two cells are almost fused together.

A mass of agglutinated spirochetes is shown in Fig. 5, Plate 11.

PLATE 11.—CULTURAL FORMS OF *Tr. avium.*

From same preparation as before, except in case of Fig. 5.

FIG. 1.—Incomplete division of a spirochete; two separate nuclei while the dark oval blepharoplast shows that it is constricted, if not actually divided.

FIG. 2.—More advanced stage of division of spirochete, showing two nuclei and two blepharoplasts.

FIG. 3.—Complete division of a spirochete. The posterior end of one cell projects beyond that of the other.

FIG. 4.—Rounding up of a spirochete.

FIG. 5.—A mass of agglutinated spirochetes from strain Y. This was obtained from a canary inoculated with strain D. The preparation was living and motile. The photograph was taken by means of instantaneous exposure. Magnification, 500 $\times$.

PLATE 1.

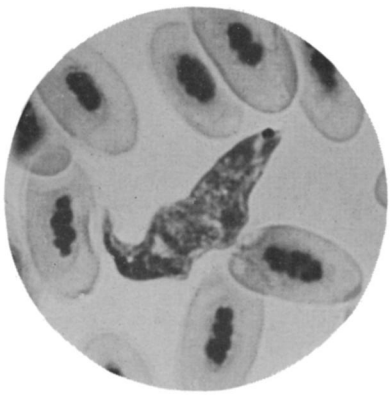


FIG. 1.

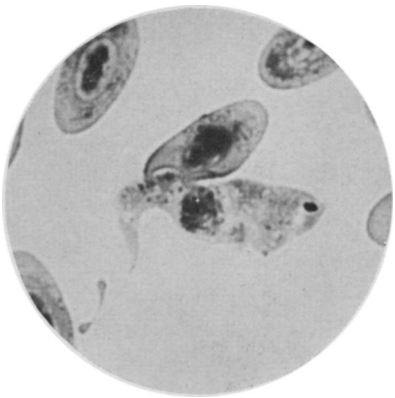
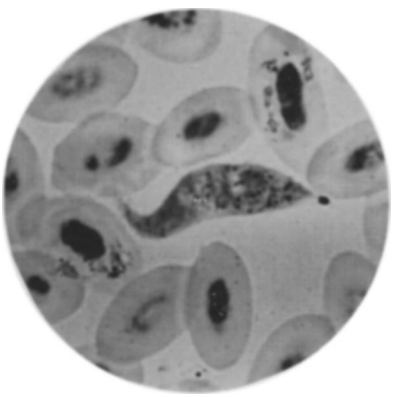


FIG. 2.



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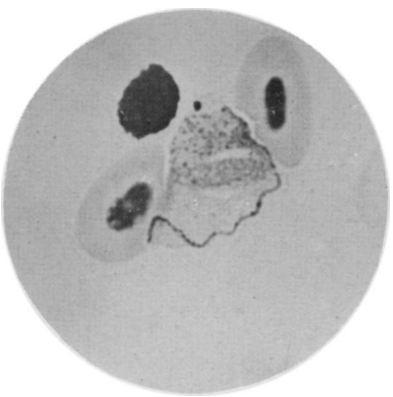


FIG. 4.

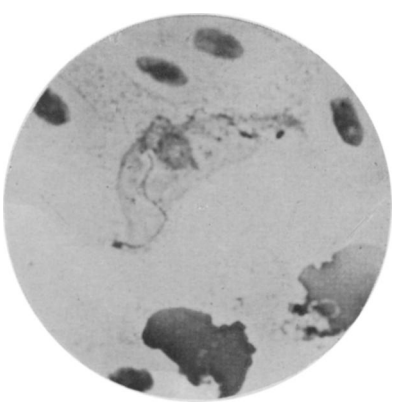


FIG. 5.

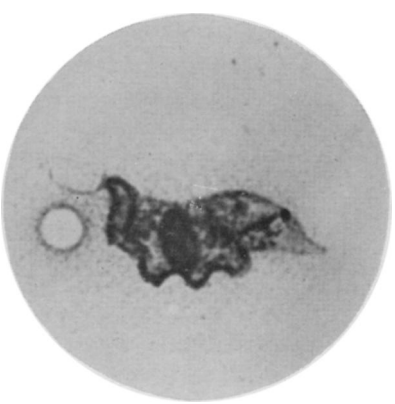


FIG. 6.

PLATE 2.

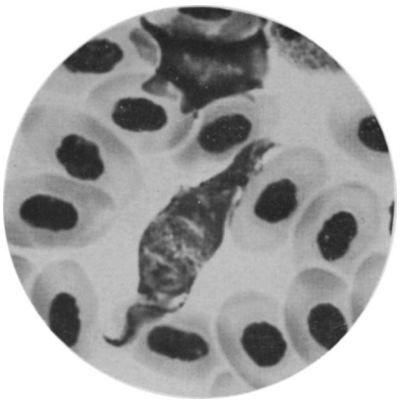
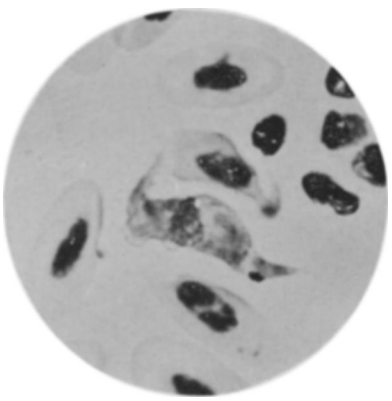


FIG. 1.

FIG. 2.



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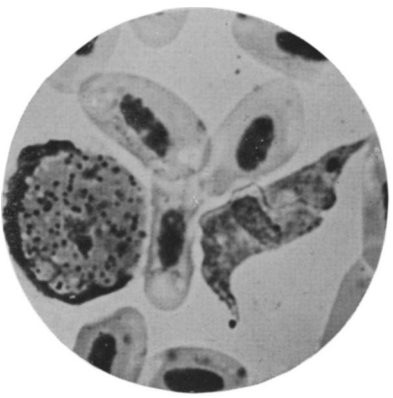

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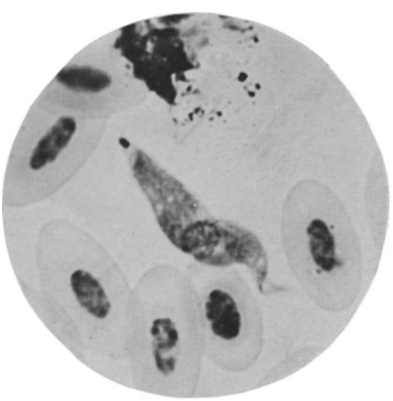


FIG. 5.

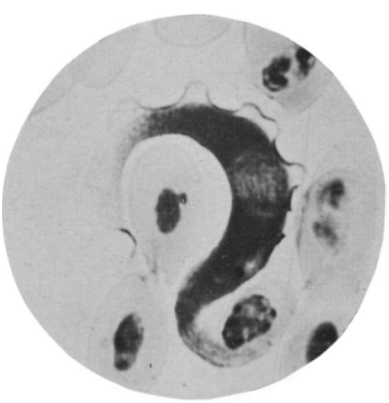


FIG. 6.

PLATE 3.

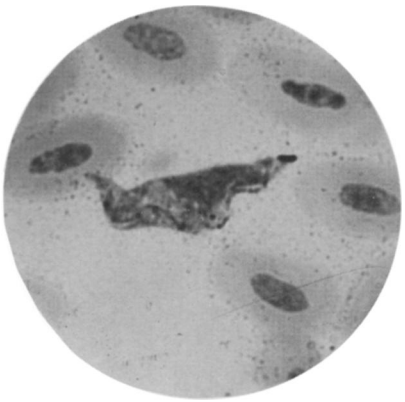


FIG. 1.

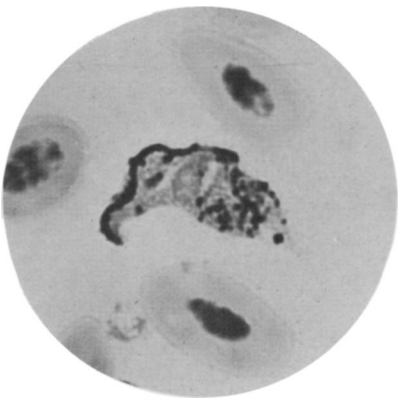


FIG. 3.

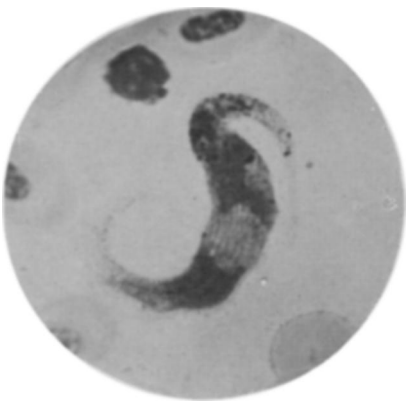
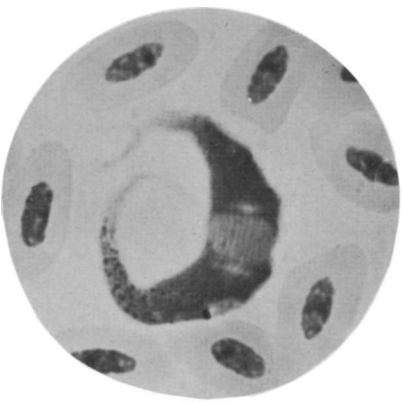


FIG. 3.



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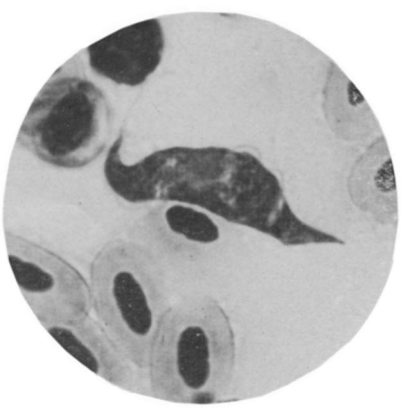

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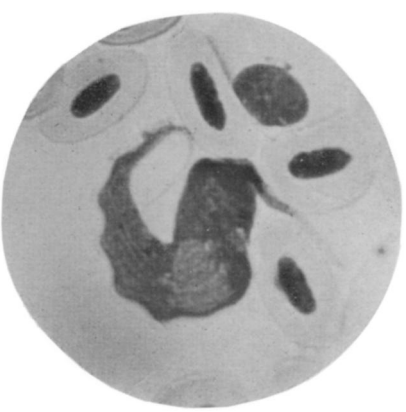


FIG. 6.

PLATE 4.

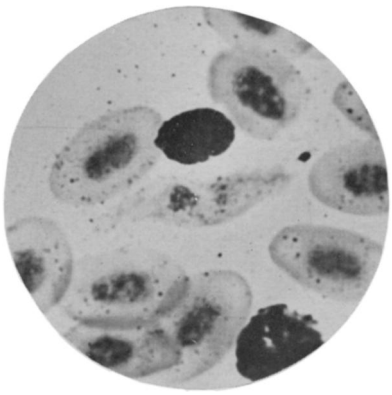
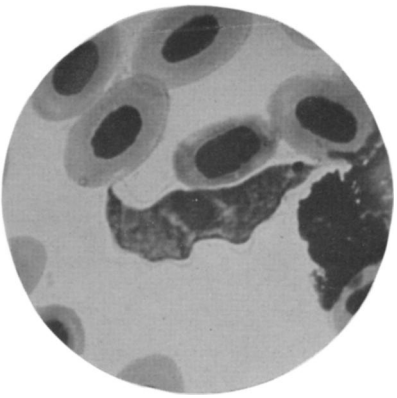


FIG. 1.



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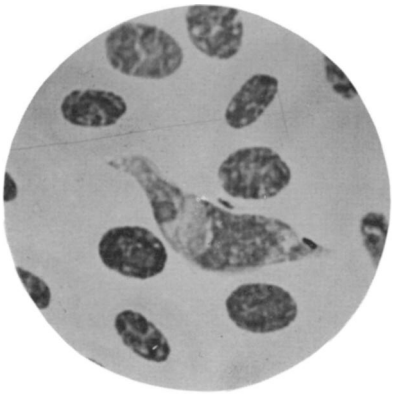

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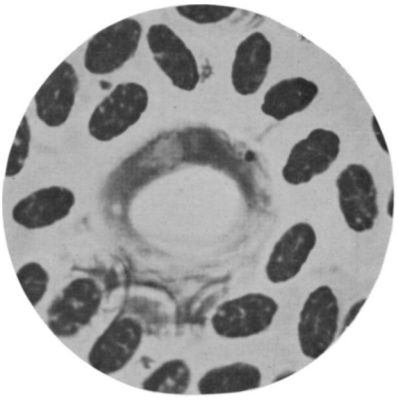

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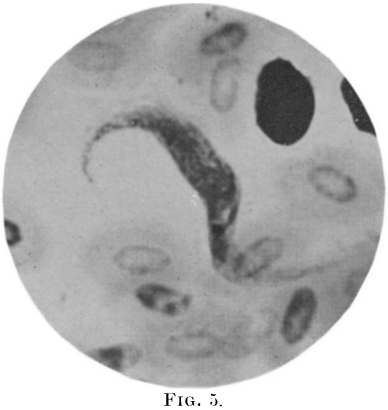


FIG. 5.

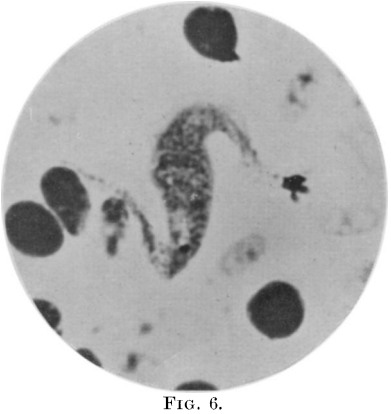


FIG. 6.

PLATE 5.

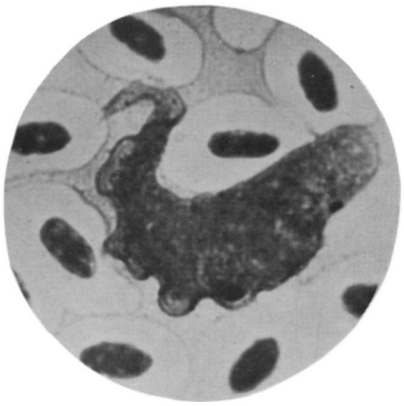


FIG. 1.

FIG. 2.

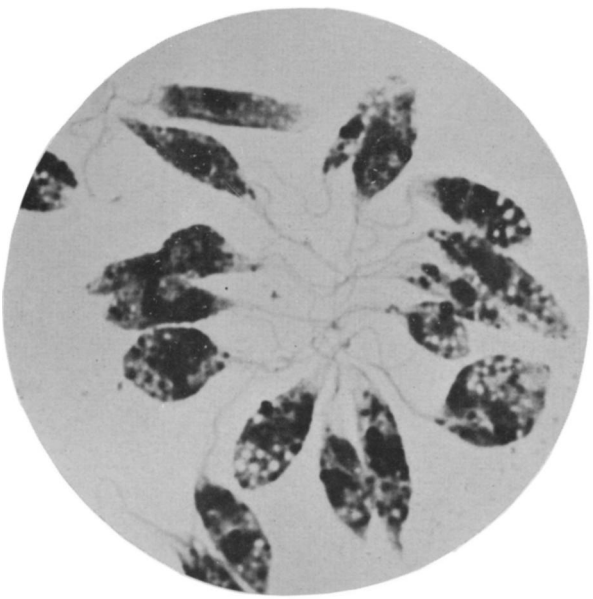


FIG. 3.

FIG. 4.

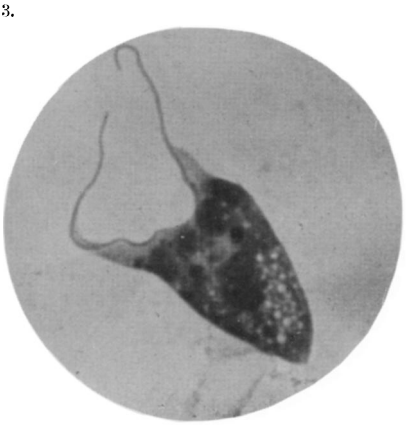


FIG. 5.

PLATE 6.

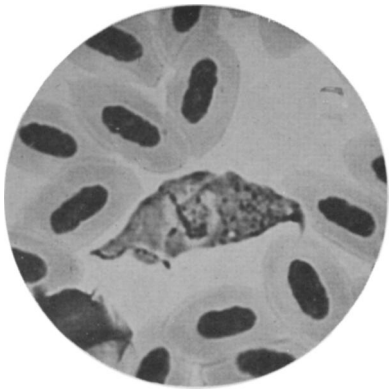


FIG. 1.

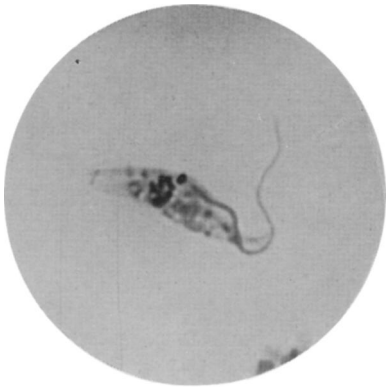


FIG. 2.

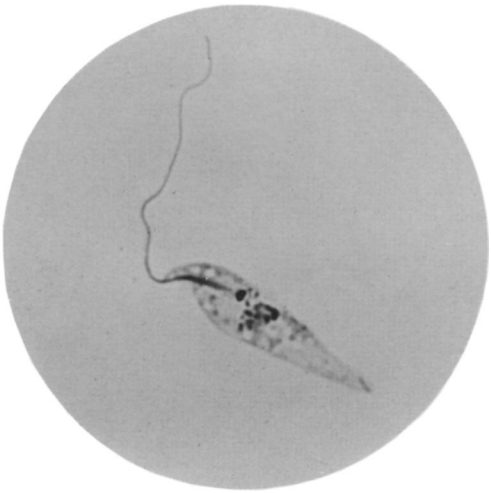
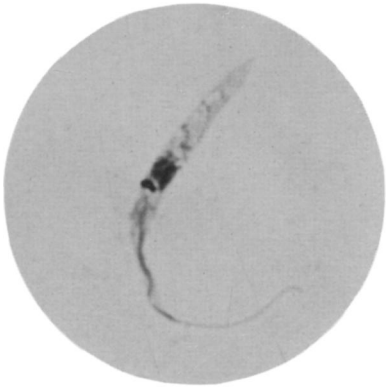


FIG. 3.



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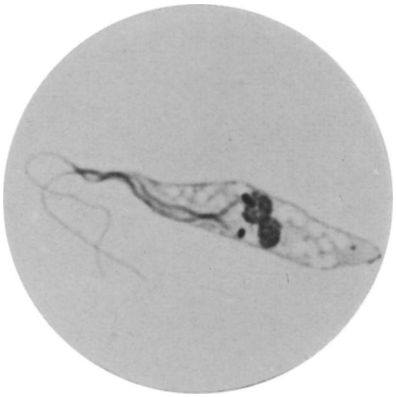


FIG. 5.

FIG. 6.

PLATE 7.

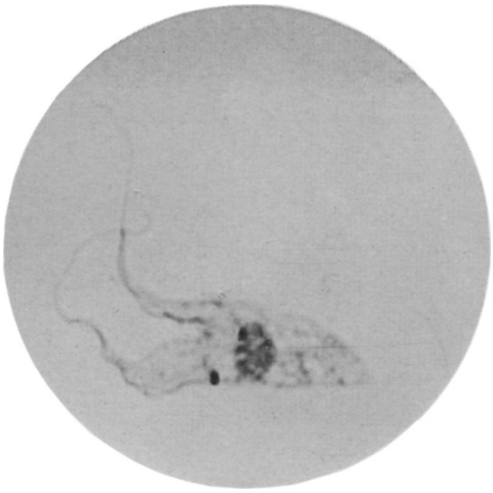


FIG. 1.

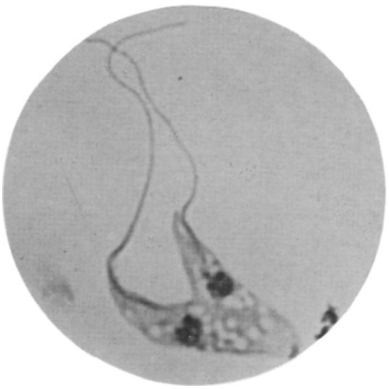


FIG. 2.

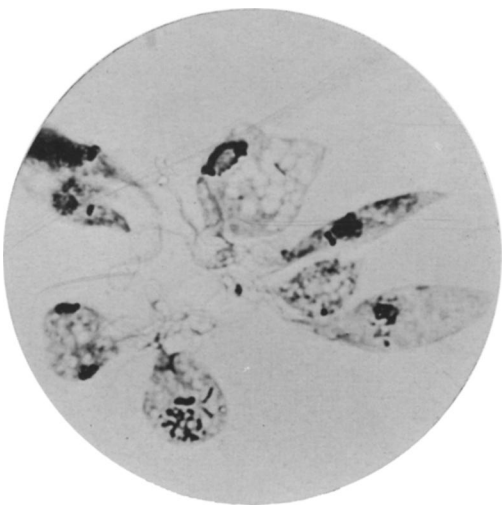
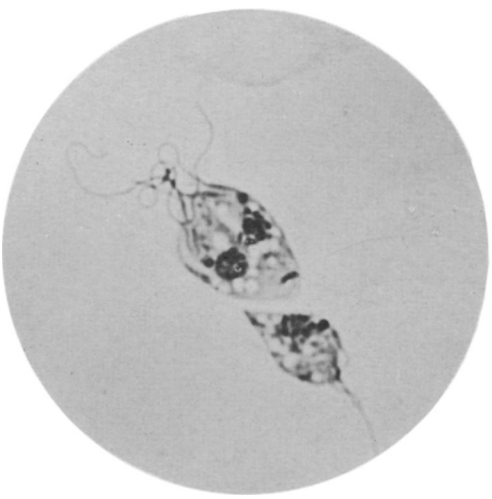


FIG. 3.



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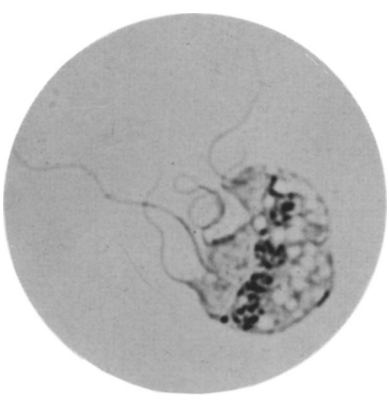


FIG. 5.

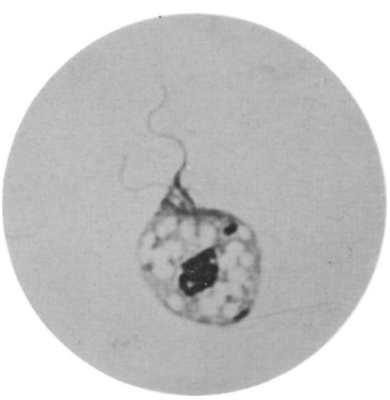


FIG. 6.

PLATE 8.

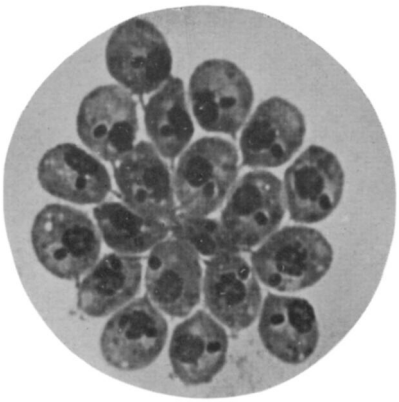


FIG. 1.

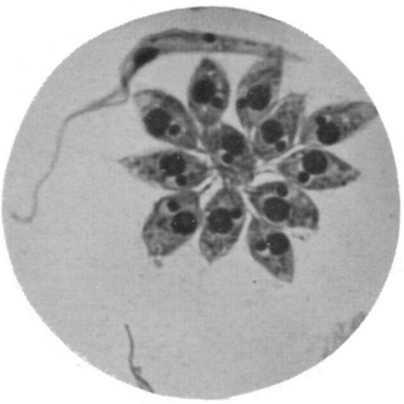


FIG. 2.

FIG. 4.

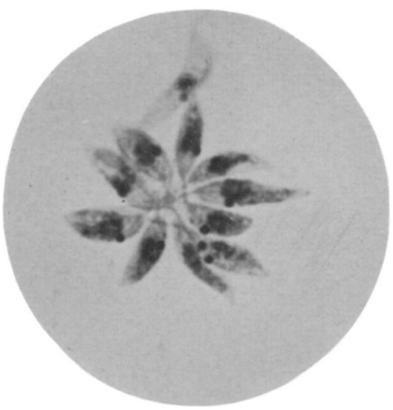


FIG. 3.

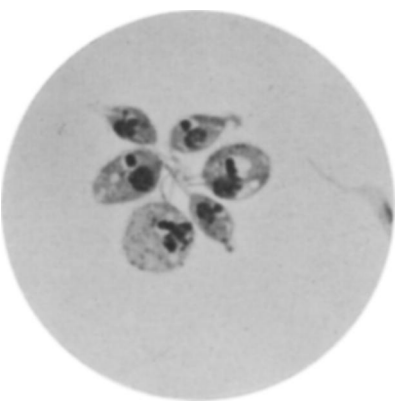


FIG. 5.

PLATE 9.

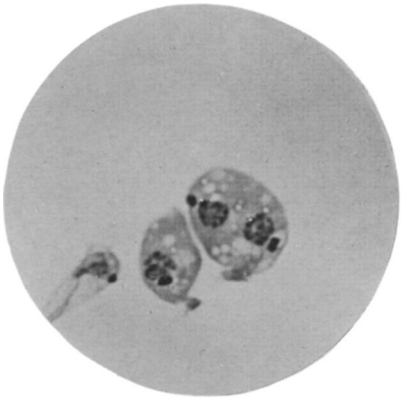
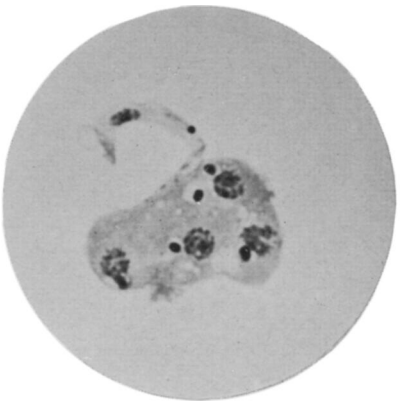


FIG. 1.



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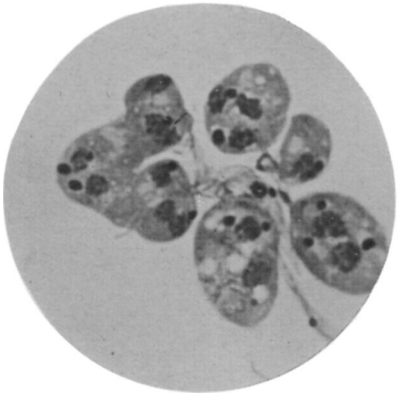

FIG. 3.

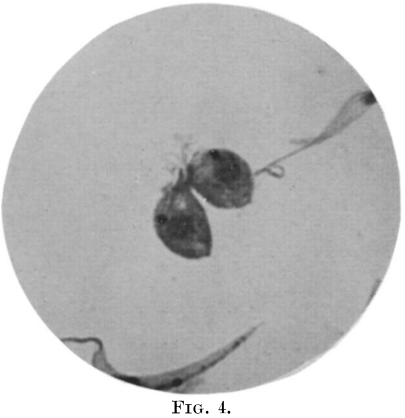

FIG. 4.

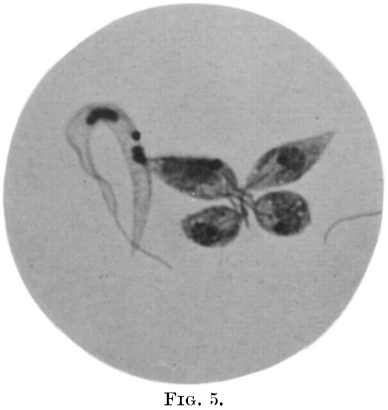

FIG. 5.

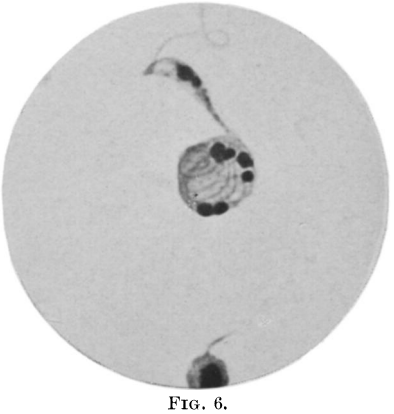

FIG. 6.

PLATE 10.

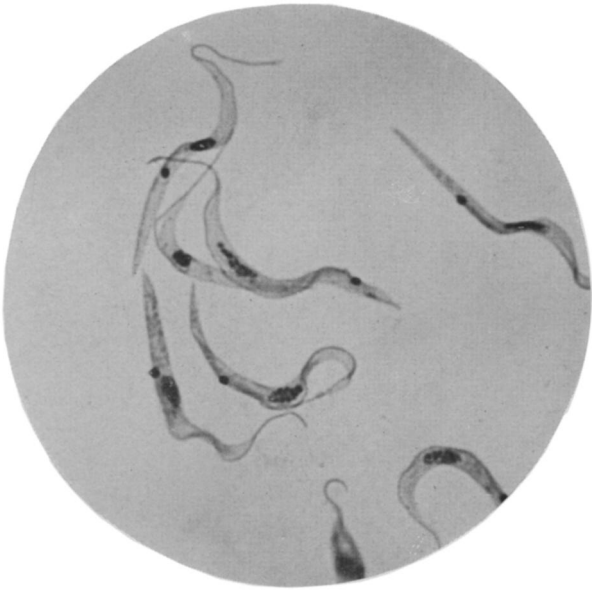

FIG. 1.

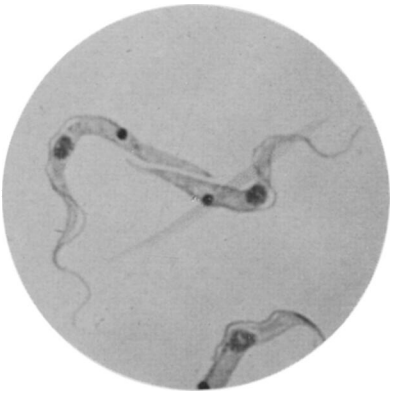

FIG. 3.

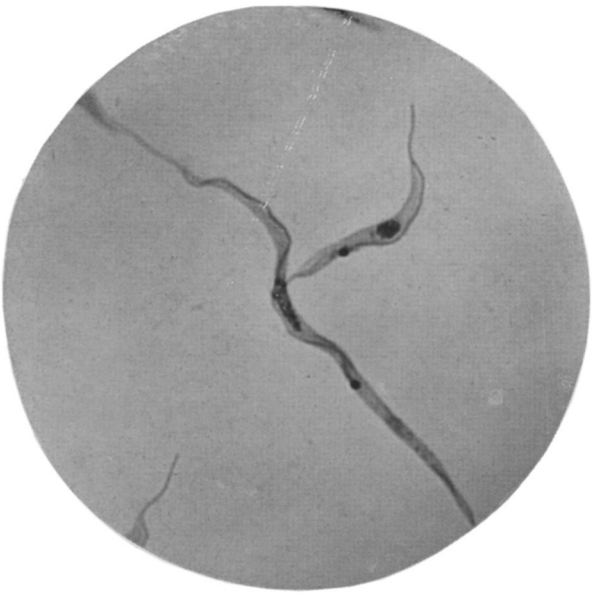

FIG. 2.

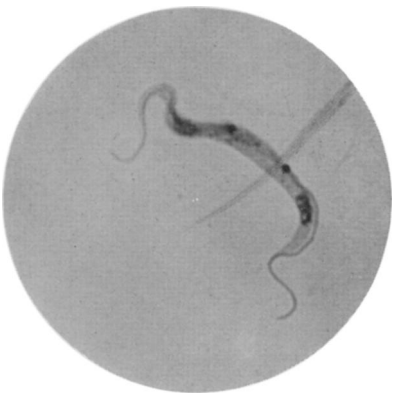

FIG. 4.

PLATE 11.

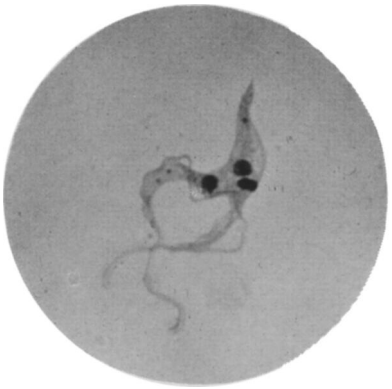

FIG. 1.

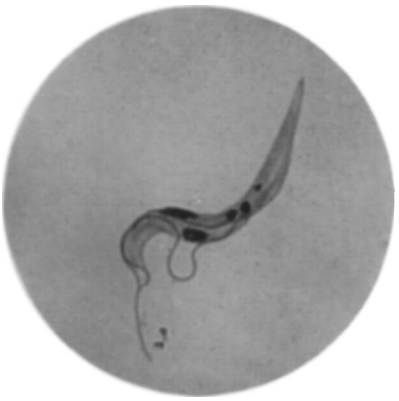

FIG. 2.

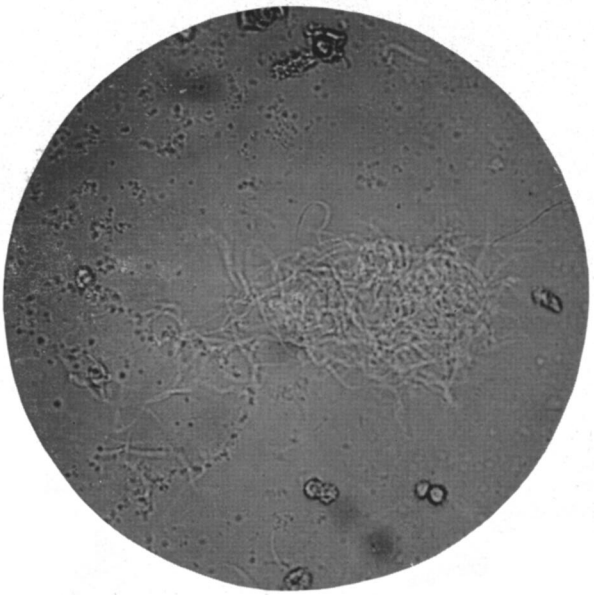

FIG. 5.

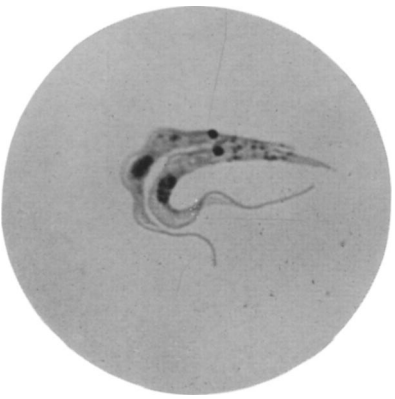

FIG. 3.

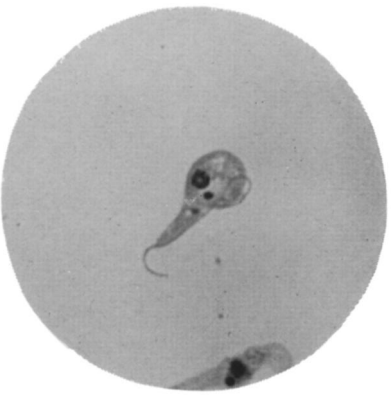

FIG. 4.